ABSTRACT

Jeffrey Edward Peacock: The Efficacy of Germicides Against Adenovirus Serotypes 2 and 8 (Under the Direction of William A. Rutala, David J. Weber, Gregory P. Samsa, Gwy-Am Shin, and Mark D. Sobsey)

Infectious diseases can be transferred from person to person via direct contact with contaminated inanimate objects. In healthcare settings, these objects can range from tabletops to bed rails to patient care equipment. One technique utilized by infection control professionals to control these environmental infectious microorganisms is disinfection. Adenovirus type 8, which commonly causes epidemic keratoconjunctivitis (EKC), is a worldwide problem in newborns and remains a significant source of morbidity and mortality in patients undergoing cataract extraction or corneal replacement procedures. One of the ways adenovirus type 8 can be spread is from patient to patient by contaminated ophthalmic equipment. Therefore, the elimination of adenovirus type 8 from inanimate objects, such as tonometers, potentially could offer significant health benefits. Unfortunately, only limited data are available on the efficacy of available germicide products versus adenovirus 8.

Given the lack of data on the efficacy of disinfection products in adenovirus eradication, this study was designed to identify which commonly used germicides were most effective in this pursuit, and in turn reduce the risk of infection resulting from patient contact with contaminated ophthalmic instruments.

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GLOSSARY OF TERMS[7, 23, 54]

Antimicrobial- an agent that is harmful to microorganisms by either killing them or inhibiting their growth

Antiseptic- a chemical germicide formulated for use on skin or tissue

Bactericide- an agent that kills bacteria

Chemical Sterilant- chemicals used for the purpose of destroying all forms of microbial life, including fungal and bacterial spores

-cidal- (i.e....virucidal, fungicidal, bactericidal, sporicidal, tuberculocidal) a suffix that identifies agents which destroy the microorganisms identified by the prefix

Cleaning- the removal of all foreign material (i.e....soil, organic material) from objects

Disinfectant- a germicide that inactivates virtually all recognized pathogenic microorganisms but not necessarily all bacterial spores on inanimate objects

Disinfection- a process that eliminates many or all pathogenic microorganisms, with the exception of bacterial spores, from inanimate objects

Germicide- an agent that destroys microorganisms, particularly pathogenic organisms

High-level Disinfection- can destroy all microorganisms, with the exception of high numbers of bacterial spores

Intermediate-level Disinfection- inactivates *Mycobacterium tuberculosis*, vegetative bacteria, most viruses, and most fungi, but it does not necessarily kill bacterial spores

Low-level Disinfection- can kill most bacteria, some viruses, and some fungi, but it cannot be relied on to kill resistant microorganisms such as tubercle bacilli or bacterial spores

Microorganism- an organism that can be seen only through a microscope. Microorganisms include bacteria, protozoa, algae, and fungi. Although viruses are not considered living organisms, they are sometimes classified as microorganisms

Nosocomial infections- a localized or systemic condition 1)that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and 2) that was not present or incubating or incubating at the time of admission to the hospital

Ophthalmic- of or relating to or resembling the eye

Pathogen- an organism which causes disease in another organism

Sterilization- the complete elimination or destruction of all forms of microbial life

Virucide- a chemical agent that destroys viruses

1. INTRODUCTION

It has been estimated that 5% of all patients admitted to hospitals will contract a hospital-acquired infection (24, 83). In the face of this threat of hospital-acquired infections in the modern healthcare setting, increasing emphasis has been placed upon infection control protocols. Traditionally, infection control professionals have focused their efforts on infection control techniques such as isolation procedures and hand-washing to prevent pathogen transmission. However, a number of recent studies have suggested that environmental surfaces also have the potential to participate in the spread of infectious agents through cross-contamination (53, 61). Pathogenic microorganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), respiratory syncytial virus (RSV), adenovirus, and rotavirus have all been isolated from environmental surfaces next to infected patients (8, 27, 53, 56). Healthcare workers and patients can then make contact with these surfaces, spreading the infectious agents by contaminated hands and medical equipment (53, 61). Therefore, these surfaces may play a role in the endemic or epidemic spread of infection (27, 53).

In late 2002, the emergence of Severe Acute Respiratory Syndrome (SARS) in southern China stimulated infection control professionals to re-evaluate their infection control plans (6). What made SARS so infamous was its ability to cause hospital outbreaks. These outbreaks affected over 100 people, including hospital staff, patients, and visitors (6, 18). The transmission of SARS appeared to be primarily by respiratory droplets over short

distances (6, 18). However in rare cases, SARS was spread by direct and indirect contact with respiratory secretions, feces, or animal vectors (6, 31, 40, 66, 82). The duration of infectivity of the SARS virus (SARS-CoV) from environmental sources remains unknown (6). However, studies on the stability of the SARS-CoV found it to be more stable at room temperature than other human coronaviruses (6, 47, 63). Other studies found that the SARS-CoV could survive for up to forty-eight hours on plastic surfaces and up to four days in diarrheal stools (6, 47, 81). Nevertheless, disinfectants were found to eliminate SARS-CoV infectivity (6, 47, 81). These observations led infection control professionals to re-examine the role of surface disinfection in their overall infection control programs.

In an effort to better understand viral inactivation and to gain further insight into another way infectious agents are controlled in healthcare settings, many new studies have focused upon surface disinfection. These surface disinfection studies have sought to determine the efficacy of various disinfectants versus common hospital pathogens found dried on environmental surfaces. Since SARS-CoV was found to be inactivated by disinfectants, naturally it is important to Infection Control Professionals (ICPs) to determine what products, in the ever-expanding cache of disinfectants, offer the greatest protection for healthcare staff and patients against other nosocomial pathogens. In this study, twenty germicides were tested to determine their efficacy versus the adenovirus, an important community and nosocomial viral pathogen. Adenoviruses cause conjunctivitis, keratoconjuntivitis, pharyngoconjunctival fever, pneumonia, and a pertussis-like syndrome (56). Adenoviruses are spread by direct contact, indirect contact, via small-droplet aerosols, by the fecal-oral route, and occasionally by ingestion of contaminated water. Nosocomially, self-inoculation from contaminated fingers and healthcare items, close contact, and fecal-oral

are the most important routes of transmission for these viruses (26, 57). Fingers can become contaminated as the result of direct contact with patients infected by the virus or by direct contact with objects that have been exposed to the virus and still contain viral residue. The risk of obtaining an adenoviral infection is greatest for those between the ages of 6 months and 5 years, but they can affect people of all ages (57).

Adenovirus serotypes 2 and 8 were the adenoviral strains chosen for investigation in this study. Adenovirus serotype 2 commonly cause enteric infections. When stools from children with gastroenteritis occurring in hospitals, outpatient clinics, and day care centers were cultured, adenovirus types 1, 2, 3, 5, 7, or 31 were isolated in 4% - 15% of all cases (57). Fever and vomiting is also common with adenovirus 2 infections. Adenovirus 2 is normally considered to be a very mild disease, but, in immunocompromised patients, it can be fatal.

In contrast, adenovirus type 8 causes eye infections, such as keratoconjunctivitis and pharyngoconjunctivial fever (57). Keratoconjunctivitis affects principally adults and the main causes are adenovirus types 8, 19, and 37 (57). Some of the main symptoms associated with adenovirus type 8 infections include eye watering, redness, discomfort, and photophobia (57). In severe cases, adenovirus 8 can cause subconjunctival hemorrhages, chemosis, or pseudomembranes (57). Common places for epidemic keratoconjunctivitis (EKC) outbreaks have included industrial plants, eye clinics and hospitals, nursing homes, camps, military bases, and child care centers (57). Transmission of adenovirus 8 can occur via the hands of medical personnel and by contaminated ophthalmic solutions and instruments, for example tonometers and slit lamps (57, 75).

Nosocomial infections of the eye caused by adenovirus type 8 are commonly viewed as insignificant. In reality though, nosocomial EKC is a worldwide problem in newborns and remains a significant source of morbidity and mortality in those patients having cataract extraction or corneal replacement procedures (75). Hospital-acquired eye infections occur at a median rate of 0.24 infections per 10,000 discharges and overall represent around 0.5% of all nosocomial infections (44, 75). It has been suggested that the lack of emphasis placed on these types of infections and the often poor documentation of their occurrence in patient records almost certainly means that these numbers appreciably underestimate the true incidence of these diseases (44).

Adenovirus 8 is extremely hardy when deposited on environmental surfaces, and it is for this reason that fomites play such an important role in transmission (75). To prevent the spread of adenovirus 8 from environmental surfaces and hospital equipment, the CDC (11, 68) and the Association of Professionals in Infection Control and Epidemiology (50) have recommended that tonometer tips used in eye clinics be cleaned with soap and water, then disinfected by soaking for 5 to 10 minutes in a solution containing either 500 ppm chlorine, 3% hydrogen peroxide, 70% ethyl alcohol, or 70% isopropyl alcohol. Even though these recommendations have been published, there are only limited data available on the efficacy of the various recommended disinfectant products versus adenovirus 8 (75). This deficit in knowledge about the efficacy of modern surface disinfectants versus adenovirus type 8 was the impetus behind this study.

2. OBJECTIVES

Given the lack of data on the efficacy of disinfection products in reducing adenovirus infectivity, this study was designed to determine the efficacies of adenovirus reduction on surfaces by commonly used germicides with particular attention focused on reducing the risk of infection resulting from patient contact with ophthalmic instruments contaminated by adenovirus type 8.

This objective was met by examining germicide efficacy as $\log_{10}$ infectivity reductions, according to the carrier test procedure employing stainless steel surfaces developed by S.A. Sattar (58), versus:

1. Adenovirus type 8 (with hard water used in disinfectant dilution)
2. Adenovirus type 2 (with hard water used in disinfectant dilution)
3. Adenovirus type 2 (with pure (sterile) water used in disinfectant dilution)
4. Adenovirus type 2 (with hard water used in dilution and the virus combined with an organic load) and germicide contact times of 1 and 5 minutes.

The germicide efficacies were then statistically compared for adenovirus serotypes 2 and 8, and for adenovirus type 2 at the different experimental conditions. The 20 germicides tested for efficacy were: Steris Sterilant 20; Cidex OPA; Cidex; Wavicide-01; Clorox (1:50); Clorox (1:10); Clorox Clean-up Cleaner; Vesphene IIse; 70% isopropyl alcohol; 70% ethanol; 3% hydrogen peroxide; Clorox disinfecting spray; Lysol brand II disinfecting spray; TBQ; Triadine; Dettol (1:20); Dettol (1:40); 4% CHG; Medicated Soft and Sure; and Acute Kare.

3. LITERATURE REVIEW

3.1 Disinfection Background

In modern infection control, a number of different techniques are employed to suppress or eradicate microbial growth in the healthcare environment in an effort to prevent the spread of infectious microorganisms. Some of the most common infection control techniques utilized to achieve this goal would include disinfection, sterilization, and cleaning. The first technique, disinfection, is defined as "a process that eliminates many or all pathogenic microorganisms on inanimate objects with the exception of the bacterial endospore" (7, 55). Disinfection is usually done by using either a liquid chemical, such as sodium hypochlorite or povidone-iodine, or by wet pasteurization (55). The degree of disinfection is dependent upon a number of factors, each of which individually can nullify or limit effectiveness of the disinfection process. Factors which can affect efficacy are: the prior cleaning of an object; the organic or inorganic debris load; the type and level of microbial contamination; the concentration of, and exposure time to, the germicide; the nature of the object, for example crevices, hinges, or lumens; and the temperature and pH of the disinfection process (55). Disinfectants can be divided into three categories based on their germicidal strength. High-level disinfectants "kill all microorganisms except high numbers of bacterial spores" (55). Intermediate-level disinfectants "may be cidal for tubercle bacilli, vegetative bacteria, most viruses and most fungi, but do not necessarily kill bacterial spores" (55). Finally, low-level disinfectants "may kill most vegetative bacteria, some fungi,

and some viruses in a practical period of time, less than ten minutes (55)." A summary of the disinfectant efficacy of high-, intermediate-, and low-level disinfectants versus various microorganisms is shown in Table 3.1.1.

Table 3.1.1: Levels of Disinfection by Types of Microorganism

	Bacteria			Fungi[a]	Viruses	
Levels	**Vegetative**	**Tubercle Bacillus**	**Spores**		**Lipid & Medium Size**	**Nonlipid & Small**
High	+[b]	+	+[c]	+	+	+
Intermediate	+	+	+/-[d]	+	+	+/-[e]
Low	+	-	-	+/-	+	+/-

[a]Includes asexual spores but not necessarily chlamydospores or sexual spores
[b]Plus sign indicates that a killing effect can be expected when the normal use-concentrations of chemical disinfectants or pasteurization are properly employed; a negative sign indicates little or no killing effect
[c]Only with extended exposure times are high-level disinfectant chemicals capable of killing high numbers of bacterial spores in laboratory tests; they are, however, capable of sporicidal activity
[d]Some intermediate-level disinfectants (e.g. hypochlorites) can exhibit some sporicidal activity; others (e.g., alcohols and phenolics) have no demonstrated sporicidal activity
[e]Some intermediate-level disinfectants although they are tuberculocidal, may have limited virucidal activity
From reference #24

The second technique used in the control of environmental infectious microorganisms is sterilization. Sterilization is defined as the "complete elimination or destruction of all forms of microbial life" (7, 55). This process is commonly performed in hospitals and other healthcare settings by physical or chemical methods. Physical sterilization can be done using several different methods: 1) steam sterilization, 2) ethylene oxide sterilization, 3) dry heat sterilization, and 4) hydrogen peroxide gas plasma (49, 55). Steam sterilization utilizes "moist heat in the form of saturated steam under pressure" and is the most common sterilization technique (55). This type of sterilization takes place in an autoclave. Microorganisms are destroyed as the steam causes coagulation and denaturing of structural proteins and critical enzymes (55). Ethylene oxide sterilization (ETO) is used when medical items cannot be steam sterilized. In this method, ETO gas is combined with carbon dioxide or hydrochlorofluorocarbon resulting in the alkylation of microbial protein, DNA, and RNA

causing microorganism death (55). The final type of physical sterilization is dry heat sterilization. This method is used only on powders, petroleum products, and sharp instruments that can be damaged by moist heat (55). A chemical sterilant is a chemical that is used to "destroy all forms of microbiological life, including fungal and bacterial spores" (55). Some examples of chemical sterilants include peracetic acid, stabilized hydrogen peroxide 6%, and glutaraldehyde-based formulations 2% (49).

Cleaning is the third process important in infectious microorganism environmental control and is defined as "the removal of all foreign material, i.e., soil, organic material, from an object's surface" (7, 55). Cleaning is carried out by the use of a combination of water and a detergent or enzymatic reagent. For disinfection or sterilization to be most effective, cleaning must precede the disinfection or sterilization process.

3.2 History of Disinfection

Chemical disinfection is a process that has existed for hundreds of years. The earliest forms of chemical disinfection evolved in Persia around 450 BC (21). To prevent drinking water from becoming unclean, the Persians used copper and silver holding vessels instead of the standard pottery containers. Elemental copper and silver both have significant antimicrobial capability, but today are not used often in disinfection processes because of their toxicity, although this is minimal with silver, and cost. Another common use of disinfectants in the ancient world was for topical treatment of wounds (21). Wine, honey, and vinegar were used for this purpose. In today's world, only vinegar, whose active ingredient is dilute acetic acid, remains a topical disinfectant in wound care where antibiotic resistant *Pseudomonas* bacteria are a problem (21). In the Middle Ages, it was common

practice among Arabs to use mercuric chloride as a wound dressing but, that chemical has subsequently fallen out of favor. Finally, in the eighteenth and nineteenth centuries, great advances were made in the field of chemical disinfection, with the introduction of new chemical disinfectants (21). Among the new chemicals introduced were copper sulfate [1767], bleaching powder [1798], creosote, from the Greek word meaning "flesh-saviour" [1836], iodine [1839], chlorine water [1843], and phenol [1860]. Many of these chemical disinfectants are still commonly used today.

3.3 Surface Disinfection

3.3.1 Spaulding Approach to Disinfection

The approach to disinfection, as it is currently practiced in health care settings, first evolved thirty years ago. This approach focuses on the disinfection and sterilization of patient- care items or equipment, and was developed by Earle H. Spaulding (55, 64). Infection control professionals find Spaulding's approach so clear and logical that it has been retained and refined, and that approach is still successfully used today. Spaulding divided health care instruments and items for patient care into three categories based on the degree of risk of infection associated with their use (64). Those three categories of patient-care items were critical, semicritical, and noncritical.

Critical patient-care items are those items that are believed to be associated with the highest risk of infection resulting from their use. All microorganisms, including bacterial spores, must be removed from critical items. By definition, critical items to contact sterile tissues or vascular tissue (55, 64). Examples of patient-care items that are placed into the critical category include surgical instruments, cardiac and urinary catheters, prosthetic device

implants, and needles (55, 64). To minimize the risk of infection from critical items, it is necessary to either purchase them as sterile or sterilize them by steam autoclaving. A chemical sterilant can be considered as an option in selected cases.

Semicritical items are those patient-care items that pose the second most significant threat for transmitting infection to patients. Semicritical items come into contact with mucous membranes and non-intact skin (55). All microorganisms, with the exception of high numbers of bacterial spores, must be removed from semicritical patient-care items. Some examples of semicritical items include respiratory and anesthesia equipment, some endoscopes, applanation tonometers, and thermometers (55). Proper disinfection of semicritical items can be achieved by using a high-level chemical disinfectant or by wet pasteurization. Some common high-level chemical disinfectants include glutaraldehyde, hydrogen peroxide, peracetic acid, peracetic acid plus hydrogen peroxide, and chlorine and chlorine-releasing compounds (55).

Finally, noncritical patient-care items are those which pose little or no threat of spreading infectious agents to patients. These types of items come into contact only with intact skin, but not mucous membranes (55). Noncritical items include bed pans, blood pressure cuffs, crutches, bed rails, linens, some food utensils, bedside tables, patient furniture, and floors (55). However, it is common belief that secondary transmission of infectious agents from noncritical items can occur. Mechanisms of spread of microbes from unclean noncritical items to patients might include transmission via the hands of health-care workers who have touched unclean items or by direct patient contact with unclean medical equipment (55, 61). Nevertheless, despite this potential risk, it is recommended to use only a low-level disinfection for noncritical items.

The simplicity of the Spaulding approach is one reason it is a favorite of infection control professionals. Its detractors, however, cite oversimplification as the greatest drawback of this approach (49, 55). The Spaulding method does not consider the problems that can result from disinfecting complicated medical equipment that is heat sensitive, or problems associated with the inactivation of certain microorganisms.

3.3.2 Surface Disinfection/Background

Inanimate environmental surfaces are considered noncritical items since they, in theory, only come into contact with intact skin and skin is considered to be a natural defense barrier to infectious agents. Surfaces are not generally viewed as important in the direct transmission of infectious agents, but, it is their role as a potential cross-contaminant, that dictates their importance in infection control. Cross-contamination can result from "acquisition of transient hand carriage by healthcare professionals due to contact with a contaminated surface, or by patient contact with contaminated surfaces or medical equipment" (53, 61). There is debate about the importance of environmental surfaces in infection control, but investigators have demonstrated such surfaces near infected or colonized patients commonly become contaminated by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), which can survive for hours or days on dry surfaces (8, 27, 53, 56). Viruses, such as respiratory syncytial virus (RSV), adenovirus, and rotavirus, can also be spread by these environmental surfaces by surface-to-finger-to-mouth or by surface-to-mouth contact. Therefore, these surfaces may contribute to endemic or epidemic spread of infection (27, 53).

Because some uncertainty exists about the exact role that environmental surfaces play in the spread of infection, the disinfection of these surfaces remains controversial. However,

the Centers for Disease Control and Prevention (CDC) recommends in their Isolation Guideline that "noncritical equipment contaminated with blood, body fluids, secretions, or excretions be cleaned and disinfected after use" (22, 53). The Isolation Guideline also states that the disinfection of bedside equipment and environmental surfaces, i.e., bed rails, bedside tables, carts, commodes, door-knobs, faucet handles, after preliminary cleaning of those items is recommended to control certain pathogens that can persist on surfaces for extended periods. Hospital floors are another surface that can be contaminated with microorganisms (53). Contamination of floors can occur by settling of airborne bacteria, by contact with shoes, wheels, etc., and by spillage. The removal of microbes from surfaces then might be viewed as an appropriate part of healthcare infection control.

3.3.3 The Surface Disinfection Controversy

Some healthcare professionals feel that the use of disinfectants on environmental surfaces is excessive and unnecessary. They argue that there is not a single study in the literature that proves that routine environmental disinfection has a positive or beneficial effect on hospital- acquired infection rates. Literature by Maki et al. is cited as evidence supporting the lack of efficacy of environmental disinfection (2, 36). Studies performed by Dharan and coworkers (17) and Ayliffe and coworkers (3, 4) demonstrate that detergents are just as effective in hospital infection control as disinfectants (2). Finally, the argument is made that the use of surface disinfectants in healthcare facilities can cause occupational diseases in housekeeping staff, such as skin irritation and allergies (2, 16, 45, 46).

In responding to these claims of lack of efficacy and potential "harm" of environmental surface disinfection, Rutala and Weber defend their recommendations for disinfection of environmental surfaces (52). Drs. Rutala and Weber offer an eight-point

argument outlining the importance of surface disinfection. First, they state that "large well-conducted trials assessing the impact of disinfectants versus detergents for environmental surfaces have not been published." Second, detergents can become contaminated and then spread bacteria throughout a patient's environment, aiding in the potential spread of pathogens to patients (4, 52, 53). Third, disinfectants are more effective than detergents at eradicating microorganisms from environmental surfaces (3, 52). Fourth, disinfectant use for cleaning surfaces contaminated with blood and potentially infectious materials is currently required by Occupational Safety and Health Agency (OSHA) guidelines (42, 52). Fifth, noncritical patient-care items including surfaces may contribute to the dissemination of infectious agents like MSRA, VRE, and viruses, i.e., rotavirus and rhinovirus, and the use of a disinfectant can prevent this environmental dissemination (8, 9, 52, 53, 56). Sixth, since noncritical patient-care items are commonly disinfected, the use of disinfectants throughout the hospital simplifies procedures for employees. Therefore, it is less likely an employee will become confused with clean-up procedures and fail to properly disinfect a surface they fail to realize has been contaminated, since all surfaces will be disinfected. Seventh, the Association for Professionals in Infection Control and Epidemiology (APIC) guidelines and CDC guidelines recommend the disinfection of environmental surfaces (24, 50, 52). Finally, and anecdotally, Dr. Weber who is the Medical Director of the Occupational Health Service at UNC Health Care System, with 5500 employees and 12,500 patient visits per year, states that he has never seen an employee with an allergic reaction to a low-level disinfectant during the nine years he has provided this service. In addition, in a Medline literature search from 1966 through March 2004, no studies were found that concluded that the use of low-level disinfectants in the hospital environment resulted in allergic reactions in exposed

health-care workers. Therefore, it is the recommendation of Rutala and Weber that, "while noncritical surfaces are not commonly associated with transmission of infections to patients, one should clean and disinfect surfaces on a regular basis" (53).

Currently, in France, Switzerland, and the USA, it is commonplace to use disinfectants in addition to detergents, for the decontamination of environmental surfaces (17). Since it is believed contaminated environmental surfaces can potentially play a role in the endemic spread of infection by cross-contamination, infection control professionals in these countries feel that, if disinfection can possibly offer any additional health benefit to patients and healthcare workers, it should be included in any environmental cleaning procedures. This is especially true when considering that any additional economic cost in time and work from disinfectant use is minimal.

3.4 Current Hospital Standards for Surface Disinfection

Current hospital disinfection procedures are standardized by various regulatory agencies, including the CDC, OSHA, and Environmental Protection Agency (EPA) (22, 24, 50, 62). A version of these recommendations as drafted by the CDC is presented in the "Guidelines for Environmental Infection Control in Health-Care Facilities" which was also written in conjunction with the Healthcare Infection Control Practices Advisory Committee (HICPAC) (62). HICPAC is a 12-member group which counsels the CDC on issues pertaining to surveillance, prevention, and control of healthcare related infections, primarily in US healthcare facilities. These CDC guidelines state that it is possible to minimize the incidence of healthcare-associated infections if certain strategies are followed in four specific areas. First, the appropriate use of cleaners and disinfectants; second, the appropriate use and

maintenance of medical equipment; third, the adherence to water-quality standards for hemodialysis, and to ventilation standards for specialized care equipment; and fourth, prompt management of water intrusion into the facility. It is the recommendations dealing with the appropriate use of cleaners and disinfectants that are of greatest relevance to this discussion. One sub-section in this strategy guide details the use of cleaners and disinfectants on surfaces that are in patient-care areas. On these surfaces, the disinfection recommendations are as follows (62):

1. Select EPA-registered disinfectants, if available, and use them according to manufacturers' instructions.
2. Do not use high-level disinfectants/liquid chemical sterilants for disinfection of either noncritical instruments and devices or any environmental surfaces
3. Follow manufacturers' instructions for cleaning and maintaining noncritical medical equipment
4. In the absence of a manufacturer's cleaning instructions follow this procedure:
    a. Clean noncritical medical equipment surfaces with a detergent/disinfectant. This may be followed by an application of an EPA-registered hospital disinfectant with or without a tuberculocidal claim (depending on the nature of the surface and the degree of contamination)
    b. Do not use alcohol to disinfect large environmental surfaces
    c. Use barrier protective coverings as appropriate for noncritical surfaces that are 1-touched frequently with gloved hands during the delivery of patient-care, 2- likely to become contaminated with blood or body substances, 3-difficult to clean

A memorandum of agreement between the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) in 1994, changed the way sterilants/high-level disinfectants are regulated (67, 69, 70). This understanding gave the FDA control of chemical germicide regulation, which was previously done by the EPA. Environmental germicides, i.e... intermediate and low-level disinfectants are still regulated by the EPA (67), giving that regulatory agency the right to make claims about range of use and antimicrobial activity (67). To be registered as a "hospital disinfectant" by the EPA, a disinfectant must

demonstrate efficacy versus three representative microorganisms... *Staphylococcus aureus, Salmonella choleraesuis,* and *Pseudomonas aeruginosa* (19, 67).

In cleaning areas that have been contaminated by spills of blood or body substances, the recommendations are as follows:

1. Use germicide registered by the EPA for use as hospital disinfectants and labeled tuberculocidal or registered germicides on the EPA List D & E (products with label claims that inactivate HIV or HBV) in accordance with label instructions to decontaminate spills of blood and other body fluids.
2. An EPA-registered sodium hypochlorite product is preferred, but if such products are not available, generic sodium hypochlorite solutions (i.e. household chlorine bleach) can be used.
    a. Use a 1:100 (500—615 ppm available chlorine) dilution to decontaminate nonporous surfaces after cleaning a spill of either blood or body fluid in patient-care settings
    b. If a spill involves large amounts of blood or body fluids, or if a blood or culture spill occurs in the laboratory, use 1:10 dilution for the first germicide.

Table 3.4.1, entitled "Tips on Surface Disinfection," is a list of steps that dental professionals can follow to ensure proper environmental cleaning in their work environment (19). Even though this table is meant for the dental worker, many of the steps listed can be generally applied to all healthcare settings.

Table 3.4.1: Tips on Surface Disinfection

Tips on Surface Disinfection
1. Cover surfaces that are difficult to disinfect. Use plastic wrap, aluminum foil, or another material impervious to water. Replace covers between patients. 2. Cover electrical switches on the chair, dental unit, and x-ray system; applying disinfectant can damage them or cause a short in the switch. 3. Use a water-based, EPA-registered disinfectant with both cleaning and disinfectant properties. Such a product provides some antimicrobial protection during the cleaning process, helps sanitize any debris spattered during the cleaning procedure, and minimizes the number of products required for surface asepsis. 4. Choose a surface disinfectant with a hydrophilic virus (rotavirus, poliovirus, hepatitis B virus) kill. 5. Follow the disinfectant manufacturer's directions for use. 6. For agents requiring dilution prior to use, use water rather than alcohol. 7. Wear heavy-duty, puncture-resistant utility gloves during surface cleaning and disinfection procedures. 8. Don protective eyewear when mixing cleaning/disinfection solutions or cleaning surfaces with a brush. 9. Wear a mask when cleaning and disinfecting to minimize inhalation and prevent direct mucous membrane contamination from spatter. *Adapted from Molinari JA. " Environmental Surface and Equipment Disinfection in Dentistry." Dent Assist 2000 Nov-Dec; 69 (6): 4-8, 10* *Reference: #19.*

3.5 Environmental Surfaces in Healthcare Settings

3.5.1 Example Environmental Surfaces

There are many types of environmental surfaces in modern healthcare facilities. The possibility exists that these environmental surfaces may play a role in transmission of infectious agents. Therefore, to decrease any exposure risks to patients and healthcare workers, infection control specialists must identify all possible surfaces on which contamination may occur. Once again, a simple and straightforward way to identify these environmental surfaces is by the Spaulding method. A modified CDC/Spaulding classification of possible contaminated surfaces can be seen in Table 3.5.1.1 (19). In 1991, the CDC modified the Spaulding approach by adding an additional category- environmental

surfaces (67). These surfaces do not come into direct contact with patients during their time in healthcare settings. Environmental surfaces can further be subdivided into medical equipment surfaces and housekeeping surfaces (67).

Table 3.5.1.1: Modified CDC/Spaulding Classification of Contaminated Environmental Surfaces

Modified CDC/Spaulding Classification of Contaminated Environmental Surfaces				
Classification	*Description*	*Dental Clinic/ Lab Examples*	*Relative Risk of Disease*	*Surface Recycling Processes*
Critical Surfaces	penetrates tissue contacts open tissue	-hand instruments -cutting instruments -burs, files, needles hand pieces, scalar tips	High	-heat sterilize -sterile, single-use disposables
Semi-Critical Surfaces	contacts mucosa	-hand instruments -mouth props -plastic prophy angles -rubber dam frames	Intermediate	-heat sterilize -single-use disposables -chemical sterilization*
Non-Critical Surfaces	contacts unbroken skin	-blood pressure cuffs -nitrous oxide face masks	Low	-sanitize with detergent (if no blood or saliva present) -intermediate level disinfection** -removable covers
Environmental Surfaces (patient care)	usually contacts healthcare personnel but not patients	-dental unit surfaces -laboratory equipment -x-ray equipment -knobs or handles on hemodialysis machines -instrument carts	Very Low	-sanitize with detergent (if no blood or saliva present) -intermediate level disinfection** -removable covers
Environmental Surfaces (housekeeping)	rarely contacts healthcare personnel or patients	-floors -walls -carpet -tabletops	Very Low	-if no obvious blood, sanitize with detergent -if blood is present, use intermediate level disinfectant**

* Only for items destroyed by heat
** Intermediate level disinfectants include iodophors, synthetic phenolics, and sodium hypochlorite
Miller CH, Palenik CJ. "Deciding how to treat various soiled surfaces." Dent Asep Rev 1995; 16: 1-4
Spaulding EH. "Chemical disinfection and antiseptics in the hospital." J Hosp Res 1972; 9: 5-31
Adapted from Molinari JA. " Environmental Surface and Equipment Disinfection in Dentistry." Dent Assist 2000 Nov-Dec; 69 (6): 4-8, 10.
Also references #19 and #55 were used

Some of the most common environmental surfaces in healthcare settings that may become contaminated are surgical equipment, hand instruments, cutting instruments, needles, catheters, thermometers, blood pressure cuffs, headrests on patient chairs, counter tops, x-ray equipment, laboratory equipment, supply containers and bottles, chair backs, faucet handles,

light switches, floors, walls, and carpets (19). Table 3.5.1.2 summarizes the environmental surfaces that are common to the dental healthcare setting. Once again though, many of these environmental surfaces are common in all healthcare settings, so this table can be applied generally.

Table 3.5.1.2: Some Surfaces Susceptible to Contamination During Patient Treatment

Some Surfaces Susceptible to Contamination During Patient Treatment	
Headrest of patient chair*	Hand piece control switches*
Counter tops	Supply containers and bottles
X-ray unit handle and cone	Evacuator control*
Bracket table	Faucet handles*
Dental team chair backs	Chair control buttons*
Light curing handle and tip*	Light switch*
Air/water syringe handle*	X-ray view box switch*
Shade guides	Hand piece hoses*
Drawer handles	Evacuator hoses*
Light handles*	Mirror handles
X-ray unit controls*	Air/water syringe hoses*
**These surfaces are usually more easily covered than precleaned and disinfected, although all operatory surfaces can be covered*	
Source: "Environmental Surface Disinfection." Dent Assist 2000 Nov-Dec; 69(6): 4-8, 10. Reference: #19	

3.5.2 Models of Environmental Surfaces in Surface Disinfection Testing

The contamination of environmental surfaces by microorganisms has been shown to be a potentially important route for microbe transmission (14, 22, 37). The presence of microorganisms on medical devices and on environmental surfaces may also be linked to an increased incidence of nosocomial infections (9, 10, 13, 14, 28, 32, 38, 67, 74, 76). It is therefore important to investigate the efficacy of various disinfectants in killing a variety of microorganisms on environmental surfaces. To determine disinfectant efficacy, a number of laboratory tests can be performed. Some types of tests that are commonly used to test disinfectant efficacy include suspension testing, surface testing, sporicidal testing, capacity

testing, and corrosion testing (73). Suspension testing places a microorganism in a liquid suspension after which disinfectant is added. The liquid is then cultured to assess microorganism survival. Surface testing or carrier testing involves drying microorganisms on a sterile surface, then applying a disinfectant. After a specified contact time, the disinfectant is washed off and the supernatant collected and cultured to determine microorganism survival. Sporicidal testing places a carrier (strip, suture, penicylinder) in a disinfectant for a specified contact time. After the contact time, the spore strip is removed, placed in an inhibitor, and assessment is performed like a surface test. The capacity test evaluates the effect adding organic matter to the test system has on disinfectant efficacy. Finally, the corrosion test is done to examine the corrosive effects that a disinfectant may have to an environmental surface. These tests can be done separately or in combinations to attain desired study results.

3.5.3 Stainless Steel Discs as a Model Environmental Surface/Inanimate Object

In studies investigating environmental disinfection, it is important to simulate as many of the common inanimate object found in practice as possible using one uniform model. Among available options, the stainless steel disc has evolved as perhaps the best representative model. Sattar et al. first proposed the use of the stainless steel disc as the model environmental surface (58). According to Sattar, there are two ways to test virucidal activities of disinfectants, either in suspension or with carrier tests. Suspension testing is the easier of the two tests to perform, but often does not provide the test agent with an optimal challenge (61). In Sattar's study (61), he found that a number of test disinfectants effectively reduced rotavirus titer in suspension testing, yet had no effect in the carrier test when the virus was dried on a non-porous surface. Since, in healthcare settings, microorganisms are

adherent to surfaces or imbedded in debris, the carrier test can better mimic these situations and thus produce more reliable data (58). When environmental surfaces are modeled for carrier tests, three types of surfaces are considered: 1) inanimate non-porous surfaces, 2) inanimate porous surfaces, and 3) skin and mucous membranes (59). Inanimate non-porous surfaces can be divided into three additional categories: 1) environmental surfaces which are treated in place and too large to soak, 2) fomites that can be immersed for disinfection, and 3) medical instruments that are heat-sensitive and must be subjected to high-level disinfection (59). Sattar acknowledges that no one model can fully represent the plethora of environmental surfaces possible in the field. But, certain considerations must be taken into account to ensure that the best carrier test model is chosen. These considerations are as follows: 1) the carrier must not bind, absorb, or sequester the test virus such that virus elution from it becomes difficult, 2) its surface should not be too smooth; instead it should be reasonably uneven to simulate topography of representative surfaces under in-use conditions, 3) if meant for reuse, it should withstand readily repeated decontamination and sterilization, 4) its surface should permit the deposition of the desired volume of the test virus as well as the test germicide and 5) the entire carrier should be submersible in a reasonably small volume of the eluent to allow efficient recovery of the virus without any wash-off as well as titration of most of the eluate (58). To meet these criteria, 1 cm diameter, #4 polished, brushed stainless steel discs were recommended as carriers. The topography of these discs is sufficiently irregular to provide a challenge to the disinfectant, yet still allow for virus elution. The stainless steel surface is also durable enough to allow for decontamination, and, at a 1 cm diameter, is large enough for deposition of the test virus and disinfectant, yet small enough to be submerged in a small volume of eluent.

3.6 Disinfectants

3.6.1 Disinfectant Types

To achieve adequate inactivation of microorganisms on environmental surfaces, thus preventing the potential spread of infectious agents, disinfectants must be used. In general, the greater the concentration of a disinfectant, the shorter the required contact time, and the better the microbial kill (49). Iodophors are the one exception to this generalization. There are a number of physical and chemical factors that can influence a disinfectant's effectiveness. These include temperature, pH, relative humidity, and water hardness (49). A number of different types of disinfectants are used in healthcare settings, and they are grouped based on their chemical properties. The main disinfectant groups for hospital-use are alcohols, aldehydes, halogens, peroxides, phenolics, and quaternary ammonium compounds (19, 21, 43, 49). These different disinfectant types and their distinguishing characteristics are shown in Table 3.6.1.1.

In 1981, the CDC Guideline for Environmental Control established many of the infection control standards for the selection and use of disinfectants in healthcare settings. Since this CDC Guideline was first published, six important changes dealing with disinfection have occurred (51): 1) formaldehyde- alcohol has been removed as a chemical sterilant/high-level disinfectant because it is toxic, irritating, and not commonly used in practice; 2) several new chemical sterilants have been added including hydrogen peroxide, peracetic acid, and peracetic acid and hydrogen peroxide in combination; 3) 3% phenolics and iodophors have been removed as high-level disinfectants because their efficacy against bacterial spores, *M. tuberculosis*, and some fungi is unproven; 4) isopropyl alcohol and ethyl alcohol have been excluded as high-level disinfectants because of their inability to inactivate

bacterial spores and because of the inability of isopropyl alcohol to inactivate hydrophilic viruses; 5) a 1:16 dilution of 2.0% glutaraldehyde-7.05% phenol- 1.20% sodium phenate has been deleted as a high-level disinfectant because of its lack of bactericidal activity in the presence of organic matter; lack of sporicidal, fungicidal, and tuberculocidal activity; and reduced virucidal activity; and 6) the exposure time required to achieve high-level disinfection has been changed from 10-30 minutes to at least 12 minutes depending on the scientific literature and FDA-approved label claim. These changes affect some of the data in Table 3.6.1.1, but much of it remains applicable to current healthcare infection control practices.

Table 3.6.1.1a: Commonly Used Hospital Disinfectant Data

Germicide	Use-dilution	Level of Disinfection	Mode of Action	Inactivates[1]					
				Bacteria	Lipophilic Viruses	Hydrophilic Viruses	*M. tuberculosis*	Mycotic Agents	Bacterial spores
Isopropyl alcohol	60-95%	Int	denaturation of proteins	+	+	-	+	+	-
Hydrogen Peroxide	3-25%	CS/High	production of hydroxyl free radicals	+	+	+	+	+	+/-
Formaldehyde	3-8%	High/Int	alkylating proteins	+	+	+	+	+	+/-
Quaternary ammonium compounds	0.4%-1.6% aqueous	Low	Enzyme inactivation; protein denaturation; cell wall disruption	+	+	-	-	+/-	-
Phenolic	0.4-5% aqueous	Int/Low	protoplasmic poison	+	+	+/-	+	+/-	-
Chlorine	100-1000 ppm free chlorine	High/Low	Enzyme inhibition; protein denaturation; inactivation of nucleic acids	+	+	+	+	+	+/-
Iodophors	30-50 ppm free iodine	Int	destruction of protein and nucleic acid structure and synthesis	+	+	+	+/-	+/-	-
Glutaraldehyde	2%	CS/High	alkylation	+	+	+	+	+	+

Table 3.6.1.1b: Commonly Used Hospital Disinfectant Data (cont'd)

	Important Characteristics									Approximate Cost ($)	
Germicide	Shelf Life >1 week	Corrosive/ Deleterious Effects	Residue	Inactivated by Organic Matter	Skin Irritant	Eye Irritant	Respiratory Irritant	Toxic	Easily Obtainable	Purchase $/gal	Cost $/gal at use-dilution
Isopropyl alcohol	+	+/-	-	+	+/-	+	-	+	+	3.70 (70%)	3.70 (70%)
Hydrogen Peroxide	+	-	-	+/-	+	+	-	+	+	24.50 (6%)	24.50 (6%)
Form-aldehyde	+	-	+	-	+	+	+	+	+	38.42 (37% wt)	3.84 (3.7% wt)
Quaternary ammonium compounds	+	-	-	+	+	+	-	+	+	10.77	.04 (0.4%)
Phenolic	+	-	+	+/-	+	+	-	+	+	9.70- 15.70	.06 (0.4%)- .08 (0.8%)
Chlorine	+	+	+	+	+	+	+	+	+	1.00 (5.25%)	.10 (0.5%)
Iodophors	+	+/-	+	+	+/-	+	-	+	+	10.10 (10%))	.05 (0.05%)
Glutar-aldehyde	+	-	+	-	+	+	+	+	+	6.50-14.00	6.50-14.00

Modified from Laboratory Biosafety Manual. World Health Organization, 1983
From Reference: #49
1; Inactivates all indicated microorganisms with a contact time of 30 min or less, except bacterial spores, which require 6-10 hr contact time
Abbreviations: Int, intermediate; CS, chemical sterilant; +, yes; -, no; +/-, variable results

3.6.2 The Ideal Disinfectant

When selecting a disinfectant product, there are certain qualities that make it ideal. It is important that an infection control specialist considers these qualities when choosing which disinfectant to use in their healthcare facility. When selecting a germicide, the ideal agent would offer rapid, broad spectrum, microbial kill; residual activity; antimicrobial activity in the presence of bioburden; minimal toxicity; and compatibility with the environmental surface being treated (19). The ideal disinfectant would also be odorless, inexpensive, and simple to use. A summary of these qualities is shown in Table 3.6.2.1. Unfortunately, no single disinfectant on the market possesses all these ideal qualities.

Table 3.6.2.1: Properties of an Ideal Disinfectant

Properties of an Ideal Disinfectant
*Broad spectrum antimicrobial activity *Active in the presence of organic matter (such as blood, sputum, and feces) and compatible with soaps, detergents, and other chemicals frequent in use *Hypoallergenic *Residual antimicrobial activity on the treated surface *Odorless or a pleasant odor *Fast-acting (ten-minute contact time or less) *Non-toxic *Surface compatibility: should not corrode instruments or metallic surfaces and not deteriorate cloth, rubber, plastic, and other materials *Easy to use, with clear label instructions *Economical *Solubility: in water *Stability: in concentrate or dilution *Cleaner: should have cleaning properties *Environmentally friendly *Source: "Environmental Surface Disinfection." Dent Assist 2000 Nov-Dec; 69(6): 4-8, 10.* *References: #19, #49 and #51 used*

3.7 Microorganisms and Environmental Surfaces/Inanimate Objects

Even with many of our modern medical advances, infectious diseases continue to place a heavy toll on human health. Both our healthcare system and our economy feel this toll. One of the places where patients are affected by infectious microorganisms is in healthcare facilities. It was estimated that in the 1970's 5% of all patients in community hospitals contracted at least one nosocomial, or hospital-acquired, infection (29). In a study conducted by the NNIS from 1987 to 1990, data collected in 79 hospitals and 196 adult and pediatric intensive care units revealed a median overall nosocomial infection rate of 9.2 infections per 100 admissions and an incidence density of 23.7 infections per 1000 patient-

days (20, 33). In a CDC study from the 1970's, the Study of the Efficacy of Nosocomial Infection Control (SENIC), nosocomial infection rates were 5.7 per 100 admissions (20, 30). However, these infection rates varied depending on the hospital type. University hospitals reported a nosocomial infection rate ranging from 6.0 to 11.5%, community teaching hospitals reported rates between 5.4 and 10.8%, and community non-teaching hospitals had a 1.4 to 7.2% nosocomial infection rate (20, 79). Approximately 4% of all nosocomial infections occur as part of an outbreak (78, 80). These outbreaks therefore are not uncommon, even though many may be preventable (78, 80). The spread of nosocomial pathogens can occur by many routes, but the most frequent may be the indirect transmission from patient-to-patient on the contaminated hands of healthcare workers (78). Hand contamination may come from exposure to inanimate sources, such as fomites or surfaces (8, 9, 35, 56, 61, 78). Therefore, when infection control specialists choose a germicide for use in a healthcare facility, it is important to consider what microorganisms are commonly found on inanimate objects. In this particular study, we focus upon viral contamination of inanimate objects. The prevention of hospital-acquired viral infections by disinfection is a desirable goal in hospital hygiene because viruses represent an important branch of nosocomial infections (26, 72, 77). It is commonly stated that nosocomial viral infections account for 5% of all nosocomial infections (1, 26, 72), but this figure may be an underestimate (1, 71). Children appear to be most susceptible to nosocomial viral infections. It has been reported that 32% of nosocomial pediatric infections were viral in etiology (1, 72). One way in which the transmission of viral microorganisms to patients from inanimate surfaces may be prevented is by surface disinfection. To achieve the goal of environmental

viral disinfection, chemical hand antisepsis, instrument and surface disinfectants must have virucidal efficacy (65).

3.7.1 Viral Survivability

It is well known that disinfectants interact differently with viruses than with bacteria. Many of these differences can be explained by the work of Klein and Deforest (34, 65). Klein and Deforest demonstrated that the presence or absence of a viral envelope and the virus's lipophilic or hydrophilic properties are the determinants of a virus's response to chemical disinfectants. Generally, enveloped viruses survive better on environmental surfaces than non-enveloped viruses (59). Other factors that can determine a virus's survival on an environmental surface are the climatic conditions, relative humidity, temperature, and visible and UV light exposure (59).

3.7.2 Nosocomial Viral Infections

Now that the factors influencing the survivability of viruses in environmental settings have been defined, it is appropriate to examine which viruses are the most common causes of infection in healthcare settings. The National Nosocomial Infection Survey (NNIS) system defines a nosocomial infection as a localized or systemic condition 1) that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and 2) that was not present or incubating at the time of admission to the hospital and for 48 hours thereafter (23). Therefore, any viral infection contracted from inanimate objects within a healthcare facility could be classified as a nosocomial infection. Viruses account for approximately 5% of all nosocomial infections, however this is most likely an underestimate (83). These infections occur most frequently in infants and children, but other groups such as the elderly, institutionalized persons of all ages, immunocompromised hosts, and patients with

underlying chronic pulmonary, renal, or cardiac disease are also highly susceptible (83). Even a viral infection usually considered inconsequential can cause significant added expenses, prolonged hospitalizations, additional diagnostic procedures, unnecessary dispensing of antibiotics, absenteeism among hospital employees, and, in some cases, appreciable morbidity and mortality (72, 83). It has been previously reported that patients infected with a nosocomial viral pathogen remain hospitalized for an average of nine days longer than uninfected controls (72). The gamut of nosocomial viral infections ranges from viremia to respiratory tract infections to enteric infections.

Respiratory viruses are one of the most common causes of nosocomial viral infections. They account for a minimum of seventy percent of all viral nosocomial illnesses (26). The rate of transmission of most nosocomial infections is constant, but the rate of transmission for respiratory viruses appears to be seasonal (1). The peak of the spread appears to be in the winter, and once again mirrors viral activity in the community. These viruses are transmitted by three mechanisms--- small-particle aerosol, self-inoculation after contact with skin or objects contaminated by viral particles, and direct inoculation by large droplets (83). Transmission by self-inoculation is typical of adenovirus and RSV (26, 83). It is common for these viruses to survive on inanimate objects for variable periods of time, from which they can then come into contact with mucous membranes, through the vehicle of contaminated hands or medical equipment, and produce infection. RSV can live on environmental surfaces for hours (26), while adenoviruses can survive for weeks to months (60). RSV is a major cause of lower respiratory tract infections in infants and children. Adenovirus infections occur principally in early childhood and can cause a variety of afflictions depending on the serotype. The major respiratory manifestations of adenovirus

include pneumonia and laryngotracheobronchitis (83). Other major nosocomial respiratory viral infections, not documented to be transmitted from environmental surfaces, include varicella zoster virus (VZV), influenza virus, parainfluenza virus, and measles.

3.7.3 Rationale for Study Virus Selection

In the United States, a variety of viruses have been commonly investigated in disinfection studies (65). Most of these viruses play a role in hospital-acquired infections. When studying the activity of disinfectants versus viruses using a carrier test setting, there must be at 2- 4 $\log_{10}$ reduction in viral titer to classify a disinfectant as effective (58, 65). In various European countries, guidelines exist defining certain viruses as acceptable test viruses for the study of disinfectants (65). In Germany, poliovirus type 1, adenovirus type 2, vaccinia virus, and papovavirus SV 40 are acceptable test viruses. In France, acceptable test viruses include poliovirus type 1, adenovirus type 5 and vaccinia virus. Other factors to consider when selecting a test virus for a germicide efficacy study would include 1) relative safety for the laboratory staff, 2) low level of biohazard containment, 3) ability to grow to titers sufficiently high for testing, 4) ability to produce cytopathic effects or plaques, or both, in cell cultures, 5) potential for nosocomial spread through contaminated environmental surfaces and medical devices, 6) member of a virus group that is known to cause outbreaks of disease in institutional settings such as hospitals, nursing homes, and day-care centers, and 7) relatively high resistance to a variety of chemical germicides (58, 60). It is also important to keep in mind that, as a general rule, enveloped viruses do not survive as well on environmental surfaces and are also more susceptible to chemical germicides. Considering these criteria, adenovirus type 2 and adenovirus type 8 were selected for use in this germicide efficacy study. (see below)

3.8 Adenovirus

Adenoviruses were first discovered in 1953 after being cultured from adenoids and tonsils surgically removed from children (57). Young children and army recruits are the groups of individuals affected most often by adenoviruses. The most common illnesses in these affected individuals are respiratory tract infections and conjunctivitis. Adenovirus infections account for 5% to 8% of all pediatric respiratory illnesses (57). Childhood diarrhea is another important illness linked with adenoviruses. Finally, these viruses are implicated as a cause of aseptic meningitis, encephalitis, hepatitis, and hemorrhagic cystitis and can cause severe disseminated infections in immunocompromised hosts (57).

3.8.1 Virology

Adenoviruses are common in nature and can affect a wide range of animal species from frogs to humans (57). There is no common antigenic determinant present throughout the entire family, but all members have a similar size, structure and polypeptide composition (57). Human adenoviruses consist of 49 different serotypes, which can be grouped into six subgroups based on immunological, biological, and biochemical characteristics (57). Generally, adenoviruses are a medium-sized virus with a diameter range of 90- 100 nm (39). Adenoviruses are a nonenveloped icosohedral virus with double-stranded DNA. It has been found that adenoviruses are unusually resistant to killing by chemical or physical agents and adverse pH conditions, permitting them to persist outside of the human body for extended periods of time (39). Table 3.8.1.1 shows a breakdown of the different human adenovirus strains. Special attention should be paid to subgroups C and D, which contain adenovirus strains 2 and 8, the focus of this study.

Table 3.8.1.1: Classification of Human Adenoviruses

Subgroup	Serotypes	HA subgroup[2]	Production of tumors in animals	% G+C in DNA
A	12, 18, 31	IV	High	47-49
B	3, 7, 11, 14, 16, 21, 34, 35	I	Weak	50-52
C	1, 2, 5, 6	III	Low or none	57-59
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42	II	Low or none	57-60
E	4	III	Low or none	57
F	40, 41	III	Low or none	57-59

1- Adapted from Shenk, T. "Adenoviridae: the viruses and their replication." *In* B.N. Fields, D.M. Knipe, P.M. Howley, eds. Fields Virology, 3rd edition. Philadelphia(PA): Lippincott-Raven; 1996. p. 2111
2- Hemagglutination (HA) subgroups are classified as follows: I, complete agglutination of monkey erythrocytes; II, complete agglutination of rat erythrocytes; III, partial agglutination of rat erythrocytes; IV, no agglutination.
Reference: #57

3.8.2 Epidemiology

Adenoviral infections occur worldwide in epidemic, endemic, and sporadic fashion. Some of the adenoviral strains shown to be endemic in certain areas of the world are serotypes 1, 2, 5, and 6, all of which are usually contracted in childhood (39). Adenovirus serotypes 8, 19, and 37 are more commonly known to cause sporadic infections and intermittent outbreaks, an example being epidemic keratoconjunctivitis (39). These ocular infection strains become endemic mostly because of poor hygienic conditions in developing countries. In the Western world, the ocular strains remain mostly epidemic and occasionally cause nosocomial outbreaks (57). In clinical settings, the adenoviral serotypes most often problematic include respiratory types in subgenus C and B, and gastroenteritis types 40 and 41 (57). The risk of obtaining an adenoviral infection is greatest for those between the ages of 6 months and 5 years, but they can affect people of all ages (57). According to the World Health Organization (WHO), serotypes 1, 2, and 5 are most commonly contracted during the first years of life, while serotypes 4, 8, and 19 are most often contracted in adulthood (57).

3.8.3 Transmission

Adenoviruses are transmitted by direct contact, via small-droplet aerosols, by the fecal-oral route, and occasionally by ingestion of contaminated water. Endemic adenoviruses in subgroup C, serotypes 1, 2, and 5, are spread by direct contact with respiratory droplets or feces (57). Self-inoculation from contaminated fingers is the most important route of transmission for these viruses. The serotype of adenovirus causing pharyngoconjunctival fever and keratoconjunctivitis, i.e. strain 8, is spread by contact with contaminated fingers or ophthalmologic instruments (57). Some of the factors that increase the risk for adenovirus transmission include close contact in crowded living areas and under low socio-economic conditions. Outbreaks have been documented in day care centers, schools, hospitals, shipyards, and military quarters (57). These adenoviral infections can be spread nosocomially via contaminated fingers and inadequately disinfected tonometers (57).

3.8.4 Clinical Manifestations

Infections caused by adenovirus occur most regularly in children between the ages of 6 months and 5 years and are manifested as upper respiratory tract infections and eye infections. Most of these infections are mild and self-contained (57). Clinical features of adenoviral infection include tonsillitis, pneumonia, acute otitis media, febrile convulsions, fever without focus of infection, laryngitis, and keratoconjunctivitis (57). In children, fever from infection lasts for approximately 5.4 days (57).

3.8.5 Test Strains

In this study, the test strains chosen for investigation were adenovirus type 2 and adenovirus type 8. Adenovirus type 2 commonly causes enteric infections. When stools from children with gastroenteritis occurring in hospitals, outpatient clinics, and day care

centers are cultured, adenovirus types 1, 2, 3, 5, 7, or 31 are isolated in 4% - 15% of all cases. Diarrhea associated with adenovirus type 2 has been known to last from 3 to 11 days, which is significantly longer than diarrhea caused by rotavirus (57). Fever and vomiting is also common with adenovirus 2 infections. Adenovirus 2 is normally considered a very mild disease, but, in immunocompromised patients, it can be fatal.

In contrast, adenovirus strain 8 can cause eye infections such as keratoconjunctivitis and pharyngoconjunctivial fever although pharyngoconjunctivitis is mostly associated with adenovirus serotypes 3 and 7 (57). Nosocomial infections of the eye caused by adenovirus type 8 are commonly viewed as insignificant. In reality though, nosocomial EKC is a worldwide problem in newborns and remains a significant source of morbidity and mortality in those patients having cataract extraction or corneal replacement procedures (75). Hospital-acquired eye infections occur at a median rate of 0.24 infections per 10,000 discharges and overall represent around 0.5% of all nosocomial infections (44, 75). It has been suggested that the lack of emphasis placed on these types of infections and the often poor documentation of their occurrence in patient records almost certainly means that these numbers appreciably underestimate the true incidence of these diseases (44).

Still, conjunctivitis is reported as one of the most common infections in the Western Hemisphere (41, 75). Epidemic keratoconjunctivitis (EKC) is the adenoviral illness most common in healthcare settings (75). When adenoviral outbreaks have occurred in healthcare facilities, infection rates have reached as high as 25% (75). Some of the main symptoms associated with adenovirus type 8 infections include eye watering, redness, discomfort, and photophobia (57). In severe cases, adenovirus 8 can cause subconjunctival hemorrhages, chemosis, or pseudomembranes (57). EKC outbreaks are not only common in eye clinics

and hospitals, and have also been documented in industrial plants, nursing homes, camps, military bases, and child care centers (57). Transmission of adenovirus 8 can occur via the hands of medical personnel and by contaminated ophthalmic solutions and instruments, for example tonometers and slit lamps (57, 75).

Adenovirus 8 is extremely hardy when deposited on environmental surfaces and inanimate objects, thus explaining why fomites and medical equipment play such an important role in transmission (75). Fifty percent of infected patients are found to have adenovirus 8 on their hands and adenovirus can be recovered from metal and plastic surfaces for more than 30 days (5, 25, 75). In the industrial and military settings, adenovirus outbreaks occur because of the use of communal bathrooms with poor hygiene. The possible persistence of adenovirus in wash basins and on hand towels for several weeks are also factors contributing to possible outbreaks. The clinical presentation of adenovirus 8 begins with conjunctivitis, chemosis, photophobia, and lacrimation, then can progress to a diffuse punctate epithelial keratitis in several days (57). This condition can resolve on its own within a two week time period, but unresolved infection can evolve to a focal subepithelial keratitis with pathognomonic corneal opacities (57). In most cases, the infection remains self-limited and the patient's eyesight is unaffected.

To prevent the spread of adenovirus 8, the CDC (11, 68) and the Association of Professionals in Infection Control and Epidemiology (50) recommend that tonometer tips be cleaned with soap and water then disinfected, by soaking for 5 to 10 minutes in a solution containing either 5000 ppm chlorine, 3% hydrogen peroxide, 70% ethyl alcohol, or 70% isopropyl alcohol. Even though these recommendations exist, there are only limited data available on the efficacy of these disinfectant products versus adenovirus 8 (75). This deficit

in knowledge about the efficacy of germicides for the eradication of adenoviruses was the driving force behind this study.

3.9 Comparison of Efficacy of Germicide Products

There have been numerous studies testing the efficacy of germicides versus various virus types. When performing virucidal testing, suspension testing and carrier testing are the two primary methods used. These methods were discussed previously in this paper, Section 3.5.2. When reviewing germicide efficacy results from previous studies, it is important to keep in mind the test method used. Sattar et al. are of the opinion that results garnered in carrier testing experiments are of higher quality than results from suspension testing (58). Results of carrier testing should also be inspected to identify what type of material was used as the carrier. In the past, frosted glass surfaces, metal screws, and plastic Petri plates and coverslips have been some of the materials used in surface disinfection testing (65). In more recent studies, carrier materials have shifted to stainless steel discs of 1 cm in diameter, following the protocol by Sattar et al (58). Another point of emphasis when judging the value of previous germicide efficacy studies is to check for the reduction in viral titer. According to J. Steinmann, a 1000-fold reduction in viral titer is required to classify any disinfectant as an effective virucidal agent (65). Sattar states that for viruses a 2-4 $\log_{10}$ reduction in infectivity titer on hard surfaces is the usual objective (58). Unfortunately, no international standard for viral titer reduction for germicide product effectiveness has been established. Therefore, variability in published results exists. In the US, germicide efficacy standards are currently regulated by the FDA. These germicide efficacy levels are generally based on the projected amounts of viral particles normally found on the healthcare items to

be disinfected. For example, effective disinfection of surgical equipment is considered to be a 5-6 $\log_{10}$ reduction in viral titer. The results from previous germicide efficacy studies can be seen in Table 3.9.1, Table 3.9.2, and Table 3.9.3.

Table 3.9.1: A Comparison of Virucidal Activity for Previously Studied Germicides

Disinfectant	Concentration	Disinfectant Type	Volume	Contact Time	Test Organism	Study Type	Log Reduction	Reference
Vesphene Iise	1:128	phenolic	80µl	0.5 min	Poliovirus	Suspension	0.033	54
Vesphene Iise	1:128	phenolic	80µl	5 min	Poliovirus	Suspension	0.22	54
TBQ	1:128	quaternary ammonium	80µl	0.5 min	Poliovirus	Suspension	0.10	54
TBQ	1:128	quaternary ammonium	80µl	5 min	Poliovirus	Suspension	0.09	54
Clorox	1:10	sodium hypochlorite	80µl	0.5 min	Poliovirus	Suspension	>3.3	54
Clorox	1:10	sodium hypochlorite	80µl	5 min	Poliovirus	Suspension	>3.3	54
Ethyl alcohol	70%	alcohol	80µl	0.5 min	Poliovirus	Suspension	0.03	54
Ethyl alcohol	70%	alcohol	80µl	5 min	Poliovirus	Suspension	0.65	54
Lysol Disinfectant	undiluted	79% ethanol, .1% quat	80µl	0.5 min	Poliovirus	Suspension	>3.3	54
Lysol Disinfectant	undiluted	79% ethanol, .1% quat	80µl	5 min	Poliovirus	Suspension	3.1	54
Lysol Antibacterial	undiluted	.1% quat	80µl	0.5 min	Poliovirus	Suspension	0.10	54
Lysol Antibacterial	undiluted	.1% quat	80µl	5 min	Poliovirus	Suspension	0.27	54
Mr. Clean	undiluted	ionic and non-ionic surfactants	80µl	0.5 min	Poliovirus	Suspension	0.19	54
Mr. Clean	undiluted	ionic and non-ionic surfactants	80µl	5 min	Poliovirus	Suspension	0.15	54
Vinegar	undiluted	-	80µl	0.5 min	Poliovirus	Suspension	0.25	54
Vinegar	undiluted	-	80µl	5 min	Poliovirus	Suspension	0.32	54
Baking Soda	undiluted	-	80µl	0.5 min	Poliovirus	Suspension	0.14	54
Baking Soda	undiluted	-	80µl	5 min	Poliovirus	Suspension	0.42	54

Reference: #54
quat: quaternary ammonium compound

Table 3.9.2: A Comparison of Virucidal Activity for Previously Studied Germicides

Disinfectant Type	Concentration	Volume	Contact Time	Study Type	99.9% Viral Titer Reduction				Reference
					CB3	HPIV3	HCV	AD5	
Sodium Hypochlorite(hypo-chlorite)	0.01%	20µl	1 min	Carrier	no	no	no	No	60
	0.1%	20µl	1 min	Carrier	no	yes	yes	No	60
	0.5%	20µl	1 min	Carrier	yes	yes	yes	Yes	60
	1%	20µl	1 min	Carrier	yes	not done	not done	Yes	60
Chloramine T (organochlorine)	0.01%	20µl	1 min	Carrier	no	yes	no	No	60
	0.1%	20µl	1 min	Carrier	no	yes	yes	No	60
	0.3%	20µl	1 min	Carrier	yes	yes	yes	Yes	60
	0.5%	20µl	1 min	Carrier	yes	not done	not done	Yes	60
Sodium hypochlorite & potassium bromide (mixed halide)	0.01%	20µl	1 min	Carrier	no	no	no	No	60
	0.05%	20µl	1 min	Carrier	no	yes	yes	No	60
	0.1%	20µl	1 min	Carrier	no	yes	yes	No	60
Povidone-iodine (iodophor)	10% (1% iodine)	20µl	1 min	Carrier	no	yes	yes	No	60
Ethanol (alcohol)	70%	20µl	1 min	Carrier	no	yes	yes	Yes	60
Glutaraldehyde	2%	20µl	1 min	Carrier	yes	yes	yes	Yes	60
Quaternary ammonium	0.04%	20µl	1 min	Carrier	no	no	no	No	60
Quat with HCL	0.04% quat 7% HCL	20µl	1 min	Carrier	yes	yes	yes	Yes	60
Quat with ethanol	0.04% quat 70% ethanol	20µl	1 min	Carrier	no	yes	yes	Yes	60
Quat with metasilicate	0.04% quat 0.5% metasilicate	20µl	1 min	Carrier	no	yes	yes	Yes	60
Chlorhexidine	0.088%	20µl	1 min	Carrier	no	yes	no	No	60
Chlorhexidine with ethanol	0.088% 70% ethanol	20µl	1 min	Carrier	no	yes	yes	Yes	60
Phenolic	0.06%	20µl	1 min	Carrier	no	no	no	No	60
Phenolic with SDS	0.06% phenolic 0.6% SDS	20µl	1 min	Carrier	no	yes	yes	No	60
Phenolic with ethanol	0.06% 70% ethanol	20µl	1 min	Carrier	no	yes	yes	Yes	60
Phenolic with SDS	0.5% phenolic 0.6% SDS	20µl	1 min	Carrier	yes	yes	yes	Yes	60

- *CB-3: coxsackievirus B3*
- *HPIV-3: human parainfluenzavirus type 3*
- *HCV: human coronavirus 229E*
- *AD-5: adenovirus type 5*
- *quat: quaternary ammonium compound*
- *Reference: #60*

Table 3.9.3: Inactivation of Viruses by Germicides

	Lowest Concentration Inactivating 10^3 to 10^7 Virus in 10 Minutes	
	Lipophilic (Adeno 2, Herpes, Vaccinia, Influenza)	**Hydrophilic** (Polio 1, Coxsackie B1, Echo 6)
Sodium hypochlorite	200 ppm	200 ppm
Iodophor	75-150 ppm [1]	150 ppm
Formalin	2%	2-8%
Glutaraldehyde	0.02%	1-2%
Ethyl alcohol	30-50%	50-70%
Isopropyl alcohol	20-50%	90% (Echo 6) 95% (neg~polio1; coxsackie B1)
Phenol	1-5%	5%
O-phenylphenol	0.12%	12% (neg)
Benzalkonium chloride	1:1000- 1:10,000	10% (neg)

Data from Klein M, DeForest A. "The chemical inactivation of viruses." Chem Spec Manuf Assoc Proc 1963:49:116-118. Reference: #34 & #49

1: variable results dependent of virus. For example, 150 ppm of iodophor are required to inactivate adenovirus 2, but 75 ppm are needed to inactivate herpes, vaccinia, and influenza

4. EXPERIMENTAL APPROACH

This germicide efficacy study was designed to gather data relating to the effectiveness of various germicide products versus adenoviruses dried onto environmental surfaces/inanimate objects. The germicides that were studied differed in their active and non-active ingredients and by their formulations as antiseptics, handwashes, hand scrubs, cleaner/detergents, disinfecting solutions, or sterilizing solutions. There were 20 potential germicide products tested: 6 antiseptics (2 of which are handwashes, 1 of which is a surgical hand scrub); 13 disinfectants (3 High-level disinfectants, 9 Intermediate-level disinfectants, and 1 Intermediate/Low-level disinfectant); and 1 chemical sterilant.

The methodology used in this experiment was adapted from Sattar et al (58). Sattar's protocol allows for germicide efficacy to be determined by simulating the drying of a viral agent onto an environmental surface, followed by treatment with various germicidal products. The $\log_{10}$ reduction of the test virus was determined from observing cytopathic effects (CPE) in liquid culture assay. The viral titer was expressed using the method of Reed and Muench (48). This experimental protocol was chosen because carrier testing is believed to produce results similar to those actually encountered in healthcare settings, as opposed to suspension testing whose results are believed to be less applicable to actual clinical practice (58). This is because viral susceptibility to germicides is dependant upon whether a virus is wet or dried. Sattar found that a number of test disinfectants effectively reduced rotavirus titer in suspension testing, yet had no effect in the carrier test when the virus was dried on a

non-porous surface (61). Since, in healthcare settings, microorganisms are adherent to surfaces or imbedded in debris, the carrier test can better mimic these situations and thus produce more reliable data (58).

The $\log_{10}$ reduction of the test organism is used as the measure of the germicide's efficacy. A comparison can then be made of the efficacy between germicide product types based on active ingredients and formulation. The efficacy of germicide types, i.e. antiseptic, disinfecting solution, chemical sterilant, can also be compared. Two different contact times were used in this study, 1-minute and 5-minutes. A germicide's efficacy can also be compared based on varying the contact time because microbial inactivation is a kinetic process that occurs over the period of time that the microbe is in contact with the antimicrobial agent. Finally, several studies were also performed to assess the sensitivity of the methodologies used, when minor experimental parameters were altered.

The only adaptations made to the Sattar protocol were as follows: 1) changing the inoculum's air drying time from 50 min to 40 min; 2) substituting glass vials for Teflon vials; and in some experimental trials 3) transferring the stainless steel discs with disinfectant, after the desired contact time, into test tubes containing neutralizers, rather than placing the neutralizer onto the disc which is covered by disinfectant. In a sub-experiment, it was shown the same amount of adenovirus type 8 was recovered from the stainless steel disc, regardless of whether a vial was used, as proposed by Sattar, or a test tube. These sub-experimental results can be seen in Appendix Table 9.5.

5. MATERIALS AND METHODS

5.1 Viral Isolates

The viral isolates utilized in these experiments were obtained from the American Type Culture Collection (ATCC, Manassas, VA): Adenovirus type 2 (ATCC strain VR 846) and Adenovirus type 8 (ATCC strain VR-1085AS/RB). The adenovirus type 8 was purchased directly from the ATCC by Dr. W.A. Rutala. The adenovirus type 2 was purchased from the ATCC by Dr. M.D. Sobsey's laboratory at The University of North Carolina at Chapel Hill for use in other experiments (35). In order to study adenovirus type 2 in these experiments, aliquots of the virus were borrowed from Dr. Sobsey's laboratory. The adenovirus type 2 obtained from Sobsey had a titer of approximately 10^8 virus particles per 1 ml. A viral titer of 10^8 virus particles per 1 ml is considered sufficiently high and ready for experimental use. To prepare a virus with a low initial titer for experimental use, it must be propagated to increase the titer and then extracted. Because the adenovirus type 8 was obtained directly from the ATCC and had not been used experimentally, it required propagation. The propagation procedure used for adenovirus type 8 was as follows:

1. Remove growth medium, 1xMEM with 10% Calf Serum and Gentamicin/Kanamycin antibiotics, from 2 T-75 cell culture flasks
2. Mix 0.1 ml of the adenovirus type 8 suspension received from the ATCC with 0.9 ml of phosphate buffered saline (PBS)
3. Inoculate 1 ml of viral suspension onto each T-75 cell culture flasks
4. Incubate the cell culture flask for 60 minutes at 37°C in 5% CO_2, allowing viral particles to absorb and infect cells
5. After 60 min, add 14 ml of 1X MEM maintenance medium onto each flask
6. Incubate plates again for 11 days
7. After incubation period, freeze (-20°C) the viral flask until time of extraction

8. The day before the viral extraction, thaw (room temperature) and freeze (-20°C) the viral flask two times to release the virus from the infected cells
9. On the morning of the extraction, thaw the viral flask again for the third time.

One additional step, viral extraction, remains before a propagated test virus is ready for use. The extraction procedure removes the viral suspension from the cell flask, liberates viruses from cells and cell debris and concentrates it. The procedure for the viral extraction was as follows:

1. After the virus has been frozen for the third time, it is thawed
2. After the cells are thawed, mix the flask, washing the dead cells off the flask surface
3. Transfer the cell suspension into a conical tube
4. Add equal volume of chloroform to the tube and vortex for 1 minute
5. After vortexing, centrifuge the tube for 15 minutes at 5000g
6. After centrifugation, withdraw the supernatant, which contains the virus. Careful is taken to not get too close to the viral/chloroform interface, so as not to contaminate the viral suspension with cell debris. Also, care is taken to not withdraw any chloroform, which is very toxic to the cell lines (Note: chloroform may also be toxic to some viruses, notably those having an envelope)
7. Once the supernatant has been removed, an add equal volume of PBS and centrifuge again
8. Withdraw supernatant and freeze (-20°C) in 1 ml aliquots. This is the virus suspension to be used in experimentation

5.2 Cell Cultures

Adenovirus type 2 and type 8 were propagated and assayed in A549 cells. These A549 cells were cultured and maintained in Eagle's minimal essential medium (1X MEM) containing 5% fetal bovine serum. The adenovirus serotypes were grown and assayed by liquid culture technique in confluent layers of A549 cells. The culture plates were 4 row, 24-well plates. The infectivity titer of the adenovirus type 2 and adenovirus type 8 strains was estimated by a quantal analysis method based on the number of infected wells as determined from observation of cytopathic effects (CPE) on inoculated cell cultures, according to the method developed by Reed and Muench (48).

5.3 Hard Water

Hard water was used when making up test germicides requiring dilution. Because the quality and disinfectant (chlorine) residual can vary in tap water at different locations, as well as at different times at the same location, standard hard water use is recommended. The use of standard hard water prevents variations in experimental results because of changes in tap water quality. A water hardness of 380-420 ppm as calcium carbonate ($CaCO_3$) was considered to be standard hard water for use in disinfectant solutions. The protocol followed to manufacture standard hard water was derived from the USEPA OPP Microbiology Laboratory. The USEPA protocol calls for two solutions, solution 1 and solution 2, to be combined with deionized water, giving an approximate hardness of 400ppm.

Preparation of Solution 1: Solution 1 is made first, combining 7.94 g of $MgCl_2$ (anhydrous) with 18.50 g $CaCl_2$ in boiled deionized water. This solution is stirred until the constituents are completely dissolved. The solution is then poured into a 250 ml flask and diluted with boiled deionized water to final volume of 250 ml. Finally, this mixture must either be autoclaved for 20 minutes at 121°C or filter sterilized using a 0.22 µm filter.

Preparation of Solution 2: Solution 2 is made by dissolving 14.01 g $NaHCO_3$ in boiled deionized water. The mixture is then poured into a 250ml flask and diluted with boiled deionized water to final volume of 250 ml. Finally, this solution is filtered using a 0.22 µm filter.

Preparation of Hard Water: The first step in this procedure is done using sterile gloves under a fume hood. First, 38.25 ml of pre-mixed solutions 1 and 2 are placed in a 10L container. The mixture is then removed from the fume hood and placed on a magnetic mixer, and a magnetic stir bar is added. Next, 8928 ml of deionized autoclaved water is placed into

the large container. Now, the "hard water" can be tested for appropriate pH. The pH must be in the range of 7.6- 8.0. Any needed adjustments to the pH can be made using 2N hydrochloric acid or sodium hydroxide. Once the proper pH is reached, the "hard water" solution must be filter sterilized again with a 0.22 μm filter. To ensure that the "hard water solution" is at the target hardness of 380- 420 ppm, any commercially available hard water testing kit can be used. In this experiment the testing kit used was manufactured by Hach® Model 5-EP mg/L #1454-01, Hardness Test Kit 20-400 mg/L.

5.4 Germicides

In this study, 20 different germicide types were tested, which varied by active ingredients, non-active ingredients, and formulation. A summary of these study germicides is shown in Table 5.4.1.

All germicide test products were made using "hard water" when dilution was required. The "hard water" was prepared according to the USEPA protocol reviewed in Section 5.3. All germicides were also made according to manufacturer's instructions, and all products were tested within the manufacturer's use-life. The germicides were stored at room temperature and those requiring dilution were made fresh each day, no more than 3 hours prior to use in experimental trials.

Table 5.4.1: Study Germicides

Germicide	Germicide Type	Active Ingredient	Formulation Tested	Classification
1) Steris Sterilant 20	Acid	35% peracetic acid	1:6100 dilution	Chemical Sterilant
2) Cidex-OPA	Aldehyde	Ortho-phthalaldehyde	Undiluted	High-level Disinfectant
3) Cidex	Glutaraldehyde	2.4% glutaraldehyde	Undiluted	High-level Disinfectant
4) Wavicide-01	Glutaraldehyde	2.65% glutaraldehyde	Undiluted	High-level Disinfectant
5) Clorox (1:50)	Halogen	6% sodium hypochlorite	1:50 dilution	Intermediate-level Household Disinfectant
6) Clorox (1:10)	Halogen	6% sodium hypochlorite	1:10 dilution	Intermediate-level Household Disinfectant
7) Clorox Clean-up Cleaner	Halogen	1.84% sodium hypochlorite	Undiluted	Intermediate-level Household Disinfectant
8) Vesphene IIse	Phenol	9.09% o-phenylphenol 7.66% p-tertiary amylphenol	1:128 dilution	Intermediate-level Disinfectant
9) 70% isopropyl alcohol	Alcohol	70% isopropyl alcohol	Undiluted	Intermediate-level Disinfectant
10) 70% ethanol	Alcohol	70% ethanol	Undiluted	Intermediate-level Disinfectant
11) Hydrogen Peroxide (3%)	Peroxide	3% hydrogen peroxide	Undiluted	Intermediate-level Disinfectant
12) Clorox Disinfecting Spray	Alcohol and QAC	65% ethanol .3% QAC	Undiluted	Intermediate-level Disinfectant
13) Lysol Brand II Disinfectant Spray	Alcohol and QAC	79% ethanol .1% QAC	Undiluted	Intermediate-level Disinfectant
14) TBQ	QAC	8% dimethyl benzyl ammonium chloride	1:128 dilution	Intermediate-/ Low-level Disinfectant
15) Triadine	Halogen	10% povidone-iodine	Undiluted	Antiseptic/ Intermediate-level Disinfectant
16) Dettol (1:20)	Pine oil and alcohol	4.8% chloroxylenol	1:20	Antiseptic/ Low-level Disinfectant
17) Dettol (1:40)	Pine oil and alcohol	4.8% chloroxylenol	1:40	Antiseptic/ Low-level Disinfectant
18) CHG 4%	-	4% chlorohexidine gluconate	Undiluted	Antiseptic/Surgical Hand Scrub
19) Medicated Soft & Sure	-	0.5% triclosan	Undiluted	Antiseptic/ Handwash
20) Acute-Kare	Phenol	1% chloroxylenol	Undiluted	Antiseptic/ Handwash

QAC: quaternary ammonium compound

5.5 Neutralization of Chemical Disinfecting Test Agents

There were multiple neutralizers used in this study to ensure that the disinfecting test agent had no more virucidal activity after the specified contact time. There are two main ways in which neutralization can be done, either dilution of the virus-germicide mixture or addition of a chemical neutralizer. In this study both techniques were utilized. Neutralization by dilution was achieved by making a 1:10 dilution of the virus-germicide mixture. The neutralization by dilution procedure was as follows:

1. Perform the virus quantitation procedure, as explained in Section 5.8, by drying adenovirus onto the stainless steel disc, followed by treatment with a test germicidal product for a specified contact time. After the contact time, 950µl 5% Fetal Calf Serum (FCS) plus chemical neutralizers are added.
2. Now that the virus-germicide mixture has been chemically neutralized, 100µl of the neutralized test formulation can be withdrawn and placed into 900µl of FCS with neutralizers.
3. This 2nd step is the neutralization by a 1:10 dilution

Chemical neutralization was done by adding chemicals to the virus-germicide mixture, which interact with the germicide to stop virucidal activity. After a specified contact time, 950µl of chemical neutralizer combined with 5% fetal calf serum (FCS) was added to the virus-germicide mixture, and the solution was vortexed for 30-60 seconds. The chemical neutralizers chosen for this study, were 3% glycine and 0.1% sodium thiosulfate. The 0.1% sodium thiosulfate solution was used to neutralize germicides #18, 4% CHG, and #7, Clorox Clean-up. Three percent glycine was the neutralizer used for the rest of the germicides. The 0.1% sodium thiosulfate was used to neutralize 4% CHG and Clorox Clean-up instead of 3% glycine, because cytotoxicity was observed when 3% glycine was used as the chemical neutralizer for these germicides. Cytotoxicity is discussed in Section 4.6.

An experiment to document the effectiveness of these neutralizers in the inactivation of the germicide products was not performed. The study design made performing an

experiment of this type difficult within the resources available for the project. The neutralization methods used in this experiment, a 1:10 dilution and chemical neutralizers, are the methods previously used successfully and recommended by Sattar (58). The 1:10 dilution and the chemical neutralizers are also commonly accepted techniques in the literature, when neutralization is required (58). The chemical neutralizers, 0.1% sodium thiosulfate and 3% glycine were recommended specifically by Dr. Sattar in a conversation held between him and our experimental team on August 30, 2003. Sodium thiosulfate is known as an effective neutralizer for chlorine products, and glycine is an amino acid known to commonly neutralize aldehydes and glutaraldehydes. In conclusion, it cannot be said with 100% certainty that all the germicide test products were rendered inactive by the neutralization techniques utilized in this study. But, the neutralization techniques used in this study have been proven effective in the previously reported literature.

5.6 Cytotoxicity Testing of Germicide Products in A549 Cells

Any toxicity that results from the germicide test formulation on the cell culture system can seriously affect the validity of study results. Subsequent cytotoxicity may not be apparent at first, i.e., producing cell degeneration, but this cytotoxicity can cause the undamaged cell layers to become unable to support viral growth. If a test disinfectant contains a fixative, the germicide may be able to kill cells without causing them to detach or produce cellular damage (58). Therefore, the germicide mixtures must be tested for cytotoxicity in A549 cells. This cytotoxicity testing was done according to the procedure of Sattar et al. (58).

Sattar explains that the importance of cytotoxicity testing is twofold: 1) it determines the dilution of the test substance that causes no apparent cytotoxicity of the cell line and 2) it assesses if the neutralizer reduces or enhances the cytotoxicity (58). The cytotoxicity testing procedure was as follows (58):

1. Make an initial 1:20 dilution and one further 10-fold-dilution of the use-dilution of the germicide in 5% FCS with and without the neutralizers.
2. Remove the culture medium from the A549 cells
3. Place 100 µl of diluted inoculum into each well of A549 cells
4. Control monolayers receive 100 µl of only 5% FCS plus neutralizers
5. Incubate the A549 cells for 45-60 minutes at 37°C with 5% CO_2
6. After this time, re-apply culture medium on A549 cells
7. Re-incubate cells for 7- 12 days and check for any visible cytotoxicity
    a. Visible cytotoxicity is seen as cells sluffing or cell detachment

Results from this study showed that the test formulation had no significant cytotoxic effects, when neutralizers were used as explained in Section 4.5. In germicide #18, 4% CHG, and germicide #19, Medicated Soft and Sure, slight cytotoxicity was observed. However, this cytoxicity was in the 10^{-1} and 10^{-2} dilutions, and therefore should have no effect on germicide efficacy results because dilutions without cytotoxicity beyond 10^{-2} were scored for virus infection

5.7 Sub-lethal Interference Testing

According to Dr. Sattar, "levels of the test substance which show no obvious cytotoxicity could still reduce or enhance the ability of the challenge virus to infect or replicate in host cells, thus interfering with the estimation of its virucidal activity" (58). Therefore, it is necessary to have a control to account for or rule out any possible interference.

The procedure used in our study for this sub-lethal interference testing control was based on Sattar et al. (58) and was as follows:

1. Quantitate the virus titer using 5% FCS as the dilutent.
    a. The serial dilutions are made as follows:
        10^{-1} = 1ml virus: 9ml FCS
        10^{-2} = 1ml 10^{-1} virus: 9ml FCS
        10^{-3} = 1ml 10^{-2} virus: 9ml FCS
        10^{-4} = 1ml 10^{-3} virus: 9ml FCS
        10^{-5} = 2ml 10^{-4} virus: 18ml FCS
        10^{-6} = 2ml 10^{-5} virus: 18ml FCS
        10^{-7} = 1ml 10^{-6} virus: 9ml FCS
2. Make test formulations using germicides #1-#20, plus a control of 5% FCS with neutralizers
    a. The test formulations were made up as: 950 µl 5% FCS with neutralizers + 50 µl germicide
3. Next, a 1:10 dilution of the test formulation was made
    a. For this 1:10 dilution use: 300 µl of test formulation was combined with 2.7 ml of 5% FCS with neutralizers
4. Maintenance media was removed from A549 cell culture plates
5. Two rows (4 wells per row) of the cell culture plates were inoculated with the 1:10 test formulation by pipeting 200 µl into each well
6. Cell culture plates were incubated for 30 min at 37°C with CO_2
7. After 30 min, each well in the first row was inoculated with 100 µl of 10^{-5} viral dilution, and each well in the second row was inoculated with 100 µl of 10^{-6} viral dilution
    a. According to previous virus quantitations the first row will be inoculated with approximately 100 virus particles, and the second row with approximately 10 virus particles
8. Incubate the cell culture plates again for 60 min at 37°C with CO_2
9. After 60 min, add 1 ml of maintenance media per well
10. Read plates for virus CPE after incubating them for 9-14 days

After performing this experiment, any significant difference observed in virus titer indicates possible interference of host cell susceptibility to the test virus from the test formulations or neutralizers. In our study, interference was found in the 1st dilution with 4% CHG, Medicated Soft and Sure, and 10% povidone-iodine. It was concluded that any interference occurring with Medicated Soft and Sure could be considered insignificant. This conclusion can be made because Medicated Soft and Sure was an "effective" germicide with a 5 minute

contact time. Therefore any results obtained in the quantitative carrier test would be considered a minimum amount of viral titer reduction compared to the overall titer reduction due primarily to the antiviral activity of the germicide. The interference observed with CHG 4% and 10% povidone-iodine was considered to be insignificant. It is our belief that this low $\log_{10}$ reduction in adenoviral titer is because CHG 4% and 10% povidone-iodine are poor virucides, not because these germicides caused changes to occur in the A549 cells. The only way to conclude this with absolute certainty though, is by performing further experiments to investigate this interference.

5.8 Quantitative Carrier Test Procedure for Testing Germicide Product Efficacy

To test the efficacy of different germicide products, a quantitative carrier test was used. This carrier test protocol was adapted from the carrier test procedure developed by Satter et al. (58). The test carrier used was the #4 polished, 1 cm diameter, stainless steel disc as recommended by Sattar. The assay chosen for viral culture technique was the liquid medium quantal assay. A549 cells were used to culture and assay the experimental viruses, adenovirus types 2 and 8. The A549 cells were grown in 24 well culture plates.

5.8.1: Main Steps in the Quantitative Carrier Test for Virucidal Activity

The procedure used is outlined in Table 5.8.1.1 (58):

Table 5.8.1.1: Quantitative Carrier Test for Virucidal Activity Protocol

1. Inoculate each carrier with 10 µl of test virus

▼

2. Allow inoculum to air dry for 40 minutes

▼

3. After drying time, place carrier in glass vial, inoculated side up

▼

4. Place 50 µl of disinfection agent on test carrier

▼

5. Allow disinfection agent to act on inoculum for specified contact time (1 or 5 minutes)

▼

6. After specified contact time, cover disc with 950 µl of 5% FCS with neutralizer

▼

7. Vortex test mixture for 30-60 seconds

▼

8. Make 1:10 dilution of test mixture by removing 100 µl of eluate and placing it into 900 µl of 5% FCS with neutralizer (This is the second neutralization step)

▼

9. Continue making 1:10 serial dilutions of eluate out to 10^{-5} dilution

▼

10. Remove maintenance medium from cell culture plates

▼

11. Inoculate serial dilutions, 10^{-1} to 10^{-5}, into cell culture plates (use 1 row per dilution and place 200 µl of each diluent into each well)

▼

12. Incubate cells for 45-60 minutes at 37°C with 5% CO_2 to allow virus to adsorb into cell monolayer

▼

13. Inoculate cell culture wells with 1X MEM maintenance media (1ml per well)

▼

14. Incubate cells for 9-14 days, then score of CPE and compute $\log_{10}$ reductions in viral titers. The $\log_{10}$ reduction of the test virus titer is obtained by observing CPE, calculating virus titers per ml using the method of Reed and Muench (47) and subtracting the $\log_{10}$ titer of treated samples from the $\log_{10}$ titer of untreated virus control samples.

5.8.2 Definition of Germicide Effectiveness

In this study, an effective germicide provides at least a 3 $\log_{10}$ reduction of adenovirus titer. This value for effecicacy was derived from various sources, as no international standard for viral titer reduction for disinfectant product effectiveness has been established. Therefore, only informal levels exist. According to Sattar, when testing

germicide efficacy versus viruses dried on inanimate objects, a 2-4 log10 reduction in infectivity titer is considered an acceptable goal (58). In the view of Steinmann, a 1000-fold or 3 $\log_{10}$ reduction in viral titer is required to classify any germicide as an effective virucidal agent (65). Dr. Rutala stated that germicide effectiveness levels are currently regulated on the national level, with the FDA being the regulatory agency in the US. These germicide efficacy levels are generally based on the amounts of viral particles normally found on the healthcare items to be disinfected. For example, effective disinfection of surgical equipment is considered to be a 5-6 $\log_{10}$ reduction in viral titer (Dr. William Rutala, personal communication). In this study, tonometers are the healthcare item whose effective disinfection is of interest. Unfortunately, it is currently unknown as to what amount of virus is actually transferred from patient to the healthcare item. According to Rutala, "it is commonly assumed though that this transfer is in the range of 3-4 $\log_{10}$ viral particles" (Dr. William Rutala, personal communication).

5.8.3 Preparation of Maintenance Media

The procedure for the preparation of the maintenance media used on A549 cells must be done aseptically, so all bottle tops should be flamed prior to opening under the hood. The solutions necessary for maintenance media preparation are 2% FCS, 100X L-Glutamine, 100X non-essential amino acids (NEAA), 100X Gen/Kan, and 100X Nystatin (NSTN), all stored at -20°C, and 100X bicarbonate, 100X Hepes buffer, and 4M $MgCl_2$, all stored at -4°C. Steps in the preparation of the maintenance media, per 100 ml of 1X MEM solution used, are outlined in Table 5.8.3.1:

Table 5.8.3.1: Preparation of Maintenance Media

1X MEM	100 ml	200 ml	300 ml	400 ml
FCS	2	4	6	8
L-G	1	2	3	4
Bicarb	1.5	3	4.5	6
Hepes	1	2	3	4
NEAA	1	2	3	4
G/K	1	2	3	4
NSTN	1	2	3	4
$MgCl_2$	.75	1.5	2.25	3
TOTAL	9.25	18.5	27.75	34

5.8.4 Preparation of Adenovirus Type 2 with 5% FCS

Adenovirus type 2 combined with 5% must be prepared for use in a sub-experiment. This sub-experiment tests how the addition of protein or organic load in the form of 5% FCS might protect adenovirus from disinfection or interact with the germicide and change its virucidal properties. To prepare the virus-organic matter suspension, 50 µl of FCS was deposited into 950 µl of 5% adenovirus. This virus-organic matter suspension was then used in the same manner as stock virus, and following the procedure in Section 5.8.1.

5.9 Statistical Analysis

To completely examine all the data obtained in the experimental trails, a statistical analysis was performed. This analysis allowed for comparison of germicide products, in order to determine under what conditions the greatest $\log_{10}$ reductions in viral titer were observed. The germicide efficacy comparisons were done as follows (Dr. Gregory Samsa, personal communication):

> "For any particular product, each pair of experimental conditions was compared by (a) pairing the results by replicate (e.g., one pair would be replicate 2 for condition 1 and replicate 3 for condition 2); (b) for each pair, determining which of the 2 conditions showed superior disinfectant properties (e.g., a log-kill of 5.0 is superior to a log-kill of 3.1, a log-kill of 5.0+ is superior to a log-kill of 3.1, the comparison between a log-kill of 5.0 and 3.1+ is indeterminate, as is the comparison between a

log-kill of 5.0+ and 3.1+); and (c) using the determinate results only, performing a conditional binomial test, with n=the number of determinate results and the probability of success under the null hypothesis equal to .50. The logic of this procedure is similar to that underlying McNemar's test.

Once these comparisons were made, p-values could be obtained. Using these p-values, it was also possible to develop confidence intervals. The confidence intervals generated were all 1-sided. The 1-sided confidence interval was chosen according to the following logic for the adenovirus type 8 data set (Dr. Gregory Samsa, personal communication):

> "Assuming that a disinfectant truly kills exactly 3.0 log of organisms, and that the observed data is symmetric around this mean of 3.0, then for 7 independent trials the probability of observing exactly 0 trials with at least 3.0 log-kill is 1/128, the probability of observing exactly 1 such trial is 7/128, ..., and probability of observing exactly 7 such trials is 1/128. Of the 11 disinfectants with an estimated mean below 2, 9 had 0 trials with at least 3.0 log-kill and 2 had 1 such trial. Accordingly, we can be confident that none of these disinfectants work well enough to be considered in practice. Similarly, for those with excellent disinfection properties, the results usually showed either 6/7 or 7/7 trials with log-kills exceeding 3.0, both of which would be unusually good results were the true log-kill actually to be 3.0 (p=.063 and p=.008, respectively). Indeed, the only disinfectant falling between the 2 extremes is the first. Recognizing that the confidence intervals are probably too conservative when most of the data show complete reduction, it might be reasonable to just declare the lower bound of the confidence interval to be 3.0 when at least 6 of the 7 trials show a log-reduction of at least this magnitude."

6. RESULTS

6.1 Adenovirus Type 2

6.1.1 Adenovirus Type 2 Disinfection using Hard Water

The experimental data for inactivation of adenovirus 2 by the germicides tested are shown in Table 6.1.1.1. These data illustrate the mean $\log_{10}$ reductions in viral titer over multiple independent trials (n= 3-5). The entire set of tables, showing data from all experimental trials with both 1 and 5 minute contact times, are included in the Appendix as Tables 9.1 and 9.2. All required germicide dilutions were done using hard water, prepared according to the protocol in Section 5.3.

Table 6.1.1.1: Effectiveness (Log_{10} Reduction) of Germicide Products Against Adenovirus Type 2 on a Stainless Steel Surface

Adenovirus Type 2 Disinfection Data (with hard water)		
Germicide	**1 Minute Contact Time Results**	**5 Minute Contact Time Results**
1) Steris Sterilant 20	Complete Reduction ≥5.2	Complete Reduction ≥6.3
2)Cidex-OPA	Complete Reduction ≥5.2	Not Done (ND)
3) Cidex	5.1	ND
4) Wavicide-01	Complete Reduction ≥5.2	ND
5) Clorox (1:50)	2.0	Complete Reduction ≥6.3
6) Clorox (1:10)	4.4	5.1
7) Clorox Clean-up	Complete Reduction ≥5.2	ND
8)Vesphene IIse	1.2	4.3
9) 70% isopropyl alcohol	2.7	3.6
10) 70% Ethanol	4.2	4.3
11) Hydrogen Peroxide (3%)	0.51	0.95
12) Clorox Disinfecting Spray	4.4	4.2
13) Lysol Disinfecting Spray	2.8	4.1
14) TBQ	0.64	1.3
15) Povidone Iodine (10%)	1.3	1.8
16) Dettol (1:20)	0.41	1.8
17) Dettol (1:40)	0.45	1.2
18) CHG 4%	0.47	0.79
19) Medicated Soft & Sure	1.1	4.4
20) Acute-Kare	0.35	1.3
*1 minute: Viral Titer Mean = $10^{-5.42}$ Viral Carrier Quantitation Mean = $10^{-5.69}$ Mean of 5 Trials * All values are Log_{10} Viral Titer Reductions		*5 minute: Viral Titer Mean = $10^{-7.66}$ Viral Carrier Quantitation Mean = $10^{-8.27}$ Mean of 3 Trials

Formulations which demonstrated at least a statistically significant 3 log_{10} reduction in adenovirus type 2 titer and thus considered to be effective germicides for the elimination of adenovirus type 2 dried on environmental surfaces/inanimate objects included: Steris Sterilant 20 (acid, p= 0.031); Cidex-OPA (aldehyde, p= 0.031); Cidex (glutaraldehyde, p= 0.031); Wavicide-01 (glutaraldehyde, p= 0.031); Clorox Clean-up (halogen, p= 0.031); 70% Ethanol (alcohol, p= 0.031); and Clorox Disinfecting Spray (alcohol with QAC, p= 0.031).

Compounds that demonstrated at least a 3 $\log_{10}$ reduction in adenovirus type 2 titer at both the 1 and 5 minute contact times, but for which these reductions were not statistically significant according to the defined criteria included: Clorox 1:10 (halogen). The statistical analysis of the germicide efficacy data can be seen in Appendix Table 9.6.

Compounds exhibiting at least a 3 $\log_{10}$ reduction in adenovirus type 2 titer with a 5 minute contact time, but less than a 3 $\log_{10}$ reduction in viral titer with a 1 minute contact time, and for which this reduction was not statistically significant were: Clorox 1:50 (halogen); Vesephene IIse (phenol); Lysol Disinfecting Spray (alcohol with QAC); 70% Isopropyl Alcohol (alcohol); and Medicated Soft and Sure (handwash). However, if allowed to interact for at least 5 minutes, these germicides may be classified as effective against adenovirus type 2.

Germicides that did not demonstrate at least a 3 $\log_{10}$ reduction in adenovirus type 2 titers with either a 1 or a 5 minute contact time, and thus considered "ineffective" against adenovirus type 2, were as follows: 3% hydrogen peroxide (peroxide); TBQ (QAC); 10% povidone-iodine (iodophor/halogen); Dettol 1:20 (pine oil with alcohol); Dettol 1:40 (pine oil with alcohol); 4% CHG (handwash); and Acute-Kare (alcohol);.

Four out of four high-level disinfectants/chemical sterilants tested were effective at eliminating adenovirus type 2 from environmental surfaces. Of the nine intermediate-level disinfectants that were tested, three were effective in the elimination of adenovirus type 2 at both 1 and 5 minutes, 1 was effective at both the one and five minute contact times, yet not statistically significant according to the established criteria and four more became effective although not statistically significant according to the established criteria, when allowed to interact with the virus for 5 minutes. In the category of low-level disinfectants and low-level

disinfectants/antiseptics, one of the antiseptics was effective when allowed to interact for 5 minutes (but not at 1 minute and not statistically significant) whereas the other five were completely ineffective.

When analyzed by active ingredients, the following categories of germicide types produced a 3-4 $\log_{10}$ reduction in adenoviral titer with a contact time of 1 and 5 minutes: peracetic acid; glutaraldehyde; aldehyde; chlorine-based halogen; alcohol; and alcohol mixed with QAC. The disinfectant types based on active ingredient that produced a 3 $\log_{10}$ reduction of adenovirus type 2 titer with contact time of 5 minutes (but not 1 minute) were as follows: chlorine-based halogen (1200ppm); phenol; alcohol mixed with QAC; alcohol; and an antiseptic/handwash. The germicide types based on active ingredient that were ineffective versus adenovirus type 2 with both 1 and 5 minute contact times were: hydrogen peroxide; QAC; iodophor (halogen); pine oil with alcohol; a phenol-based handwash; and a surgical handscrub.

6.1.2 Adenovirus Type 2 Disinfection Using Pure (Sterile) Water

The effect of water type used in the dilution of germicides on surface disinfectant efficacy was analyzed by comparing the efficacy of dilutions made with hard water to those made with pure (sterile) water. A 1-minute surface contact time for each germicide was used to inactivate adenovirus type 2 in this sub-experiment. No data were collected using a 5-minute contact time per disinfectant. The results are shown in Table 6.1.2.1. The statistical analysis of the germicide efficacy data is shown in Appendix Table 9.6.

Table 6.1.2.1: Effectiveness of Germicide Products Against Adenovirus Type 2, when Pure (sterile) Water is used for Dilutions instead of Hard Water

Adenovirus Type 2 Disinfection Data (with pure [sterile] water)			
Germicide	**1 Minute Contact Time Results**		
	Trial 1	Trial 2	Mean
1) Steris Sterilant 20	3.3	Complete Reduction ≥6.6	5.0
2) Cidex-OPA	Complete Reduction ≥5.0	Complete Reduction ≥6.6	Complete Reduction ≥5.8
3) Cidex	Complete Reduction ≥5.0	Complete Reduction ≥6.6	Complete Reduction ≥5.8
4) Wavicide-01	Complete Reduction ≥5.0	Complete Reduction ≥6.6	Complete Reduction ≥5.8
5) Clorox (1:50)	1.0	3.4	2.1
6) Clorox (1:10)	Complete Reduction ≥5.0	Complete Reduction ≥6.6	Complete Reduction ≥5.84
7) Clorox Clean-up	4.0	Complete Reduction ≥6.6	5.3
8)Vesphene IIse	0.7	1.1	0.94
9) 70% isopropyl alcohol	3.0	2.0	2.5
10) 70% Ethanol	3.3	2.3	2.9
11) Hydrogen Peroxide (3%)	0.7	1.1	0.94
12) Clorox Disinfecting Spray	Complete Reduction ≥5.0	3.1	4.0
13) Lysol Disinfecting Spray	3.0	2.6	2.8
14) TBQ	1.3	1.1	1.2
15) Povidone Iodine (10%)	0.7	1.1	0.94
16) Dettol (1:20)	0.7	1.1	0.94
17) Dettol (1:40)	No Reduction	1.1	0.59
18) CHG 4%	No Reduction	1.1	0.59
19) Medicated Soft & Sure	0.5	1.1	0.84
20) Acute-Kare	No Reduction	1.1	0.59
*Trial 1: Viral Titer mean = $10^{8.5}$ Carrier Quantitation mean = $10^{-5.0}$ *All values are Log_{10} Viral Titer Reductions		*Trial 2: Viral Titer = $10^{8.67}$ Carrier Quantitation = $10^{-6.67}$	

The use of pure water instead of hard water in germicide dilution appeared to have no impact on the effectiveness of the germicide products in the $\log_{10}$ reduction of adenovirus type 2. For example, when pure water, rather than hard water was used in the dilution of product X, the $\log_{10}$ reductions in viral titer differed by an average of 0.62 $\log_{10}$. In no instance did the classification of the effectiveness of a germicide change because of the use

of pure water instead of hard water. When analyzed for statistical significance, the data did not show that the $\log_{10}$ reductions of adenovirus type 2 were greater when pure (sterile) water was used in dilution, instead of hard water (Appendix Tables 9.7-9.9).

6.1.3 Adenovirus Type 2 Disinfection Using Hard Water and Combined with 5% FCS

Another set of experiments was done to investigate how the addition of protein or organic load in the form of FCS might protect adenovirus type 2 from disinfection. In this experiment, a 5% FCS-adenovirus mixture was created, and all required germicide dilutions were done using hard water. The results from the efficacy testing of the germicide test products prepared with hard water versus adenovirus 2 suspended in 5% FCS can be seen in Table 6.1.3.1. The statistical analysis of this germicide efficacy data is shown in Appendix Tables 9.6.

Table 6.1.3.1: Effectiveness of Germicide Products Against Adenovirus Type 2, using Hard Water Containing 5% FCS

Adenovirus Type 2 Disinfection Data (using hard water and organic load)						
Germicide	1 Minute Contact Time Results			5 Minute Contact Time Results		
	Trial 1	Trial 2	Mean	Trial 1	Trial 2	Mean
1) Steris Sterilant 20	5.6	Complete Reduction >5.0	5.3	ND	ND	ND
2) Cidex-OPA	4.6	Complete Reduction >5.0	4.8	ND	ND	ND
3) Cidex	Complete Reduction >6.3	Complete Reduction >5.0	Complete Reduction >5.6	ND	ND	ND
4) Wavicide-01	Complete Reduction >6.3	Complete Reduction >5.0	Complete Reduction >5.6	ND	ND	ND
5) Clorox (1:50)	ND	1.3	1.3	1.8	2.6	2.2
6) Clorox (1:10)	2.6	Complete Reduction >5.0	3.8	ND	ND	ND
7) Clorox Clean-up	Complete Reduction >6.3	Complete Reduction >5.0	Complete Reduction >5.6	ND	ND	ND
8)Vesphene IIse	0.8	ND	0.8	0.8	0.8	0.8
9) 70% isopropyl alcohol	3.0	0.5	1.7	4.6	3.3	3.9
10) 70% Ethanol	5.0	2.0	3.5	Complete Reduction >6.3	5.6	5.9
11) Hydrogen Peroxide (3%)	0.8	0.33	0.57	0.8	0.8	0.8
12) Clorox Disinfecting Spray	3.3	2.0	2.6	Complete Reduction >6.3	Complete Reduction >6.3	Complete Reduction >6.3
13) Lysol Disinfecting Spray	3.0	1.7	2.3	5.0	Complete Reduction >6.3	5.6
14) TBQ	0.8	No Reduction	0.4	0.8	0.8	0.8
15) Povidone Iodine (10%)	1.0	0.7	0.85	0.8	0.8	0.8
16) Dettol (1:20)	0.8	0.33	0.57	0.8	0.8	0.8
17) Dettol (1:40)	0.8	0.33	0.57	0.8	0.8	0.8
18) CHG 4%	0.8	No Reduction	0.4	0.8	0.8	0.8
19) Medicated Soft & Sure	0.8	No Reduction	0.4	0.8	0.8	0.8
20) Acute-Kare	0.8	No Reduction	0.4	0.8	0.8	0.8
* Trial 1 Viral Titer = $10^{>9.5}$ Carrier Quantitation = $10^{6.3}$ * Trial 2 Viral Titer = $10^{8.0}$ Carrier Quantitation = $10^{5.0}$ * All values are Log_{10} Viral Titer Reductions				*Trial 1 Viral Titer = $10^{>9.3}$ Carrier Quantitation = $10^{6.3}$ *Trial 2 Viral Titer Mean = $10^{8.3}$ Carrier Quantitation Mean = $10^{6.3}$		

Under these test conditions, the following germicides can be classified as "effective" virucides, regardless of whether a 1 or 5 minute contact time is used, but this effectiveness was not statistically proven with 95% confidence: Steris Sterilant 20 (acid); Cidex OPA (aldehyde); Cidex (glutaraldehyde); Wavicide-01 (glutaraldehyde); Clorox 1:10 (halogen); Clorox Clean-up (halogen); and 70% ethanol (alcohol).

Germicides classified as "effective" when a 5 minute contact time was used, but "ineffective" for only a 1 minute contact time included: 70% isopropyl alcohol (alcohol); Clorox Disinfecting Spray (alcohol and QAC); and Lysol Disinfecting Spray (alcohol and QAC).

Germicides unable to achieve a 3 $\log_{10}$ reduction at either a 1 or 5 minute contact time (i.e., "ineffective") were Clorox 1:50 (halogen); 3% Hydrogen Peroxide (peroxide); TBQ (QAC); 10% Povidone Iodine (halogen); Dettol 1:20 (pine oil and alcohol); Dettol 1:40 (pine oil and alcohol); CHG 4% (handwash); Medicated Soft and Sure (handwash); and Acute-Kare (phenol).

6.1.4: A Comparison of Germicide Efficacy Against Adenovirus Type 2 Dried on Environmental Surfaces, with Variable Conditions

Examining data from the two experiments with variable water quality conditions (the use of pure (sterile) water in germicide dilution instead of hard water and the addition of organic matter to the virus suspension) made it possible to analyze the effects of these variable conditions. The results of these experiments as compared to the germicide efficacy data with hard water only are presented in Table 6.1.4.1. These results were obtained using only a 1-minute germicide contact time. A statistical analysis done to compare the $\log_{10}$ reductions obtained when the experimental conditions for water quality were varied is shown in Appendix Tables 9.7- 9.9.

Table 6.1.4.1: A Comparison of Germicide Efficacy Data versus Adenovirus Type 2 Dried on Environmental Surfaces, with variable conditions

Adenovirus Type 2 Disinfection Data (Comparison of Disinfectant Efficacy with Variable Conditions)			
Germicide	**1 Minute Contact Time Results**		
	Hard Water	Pure (sterile) Water	Hard Water and Organic Load
1) Steris Sterilant 20	Complete Reduction ≥5.2	5.0	5.3
2) Cidex-OPA	Complete Reduction ≥5.2	Complete Reduction ≥5.8	4.8
3) Cidex	5.0	Complete Reduction ≥5.8	Complete Reduction ≥5.6
4) Wavicide-01	Complete Reduction ≥5.2	Complete Reduction ≥5.8	Complete Reduction ≥5.6
5) Clorox (1:50)	1.9	2.1	1.3
6) Clorox (1:10)	4.4	Complete Reduction ≥5.8	3.8
7) Clorox Clean-up	Complete Reduction ≥5.2	5.3	Complete Reduction ≥5.6
8)Vesphene IIse	1.2	0.94	0.8
9) 70% isopropyl alcohol	2.7	2.5	1.7
10) 70% Ethanol	4.2	2.8	3.5
11) Hydrogen Peroxide (3%)	0.51	0.94	0.57
12) Clorox Disinfecting Spray	4.4	4.0	2.6
13) Lysol Disinfecting Spray	2.8	2.8	2.3
14) TBQ	0.64	1.2	0.4
15) Povidone Iodine (10%)	1.3	0.94	0.85
16) Dettol (1:20)	0.41	0.94	0.57
17) Dettol (1:40)	0.45	0.59	0.57
18) CHG 4%	0.47	0.59	0.4
19) Medicated Soft & Sure	1.1	0.84	0.4
20) Acute-Kare	0.35	0.59	0.4
	*Mean Results (n=5) from Table 6.1.1.1	*Mean Results (n=2) from Table 6.1.2.1	*Mean Results (n=2) from Table 6.1.3.1
*All values are Mean Log_{10} Viral Titer Reductions			

The use of pure water instead of hard water for germicide dilution appeared to have no impact on the effectiveness of the germicide products in the log_{10} reduction of adenovirus type 2. For example, when pure water was used rather than hard water in the dilution of the test product, the log_{10} reductions in viral titer differed by an average of 0.62 log_{10}. In no

instance did the classification of the effectiveness of a germicide change due to the use of pure water instead of hard water. The only change in germicide effectiveness was in 70% ethanol which changed from an effective classification in the hard water trial, to ineffective classification in the pure water trial. This change contradicts the average change seen throughout the rest of the germicide test products however, and was not related to the type of dilution water used because this disinfectant product required no dilution.

The addition of organic matter into viral suspension resulted in a decrease in the effectiveness of viral titer reduction of two test germicide products. The reduction in viral titer by 70% isopropyl alcohol and Clorox disinfecting spray was reduced by 1- 1.5 $\log_{10}$ when adenovirus type 2 was in the presence of organic matter. This loss of virucidal activity resulted in Clorox disinfecting spray being reclassified as "ineffective" and 70% isopropyl alcohol as being reclassified as "ineffective".

When these conditions were analyzed statistically for all germicides tested, it was found that the $\log_{10}$ reductions of viral titer obtained from the use of pure (sterile) water in dilution were not significantly greater than the $\log_{10}$ reductions obtained when hard water was used in dilution. The $\log_{10}$ reductions in viral titer differed by an average of 0.62 $\log_{10}$. The $\log_{10}$ reductions obtained when hard water was used in dilution were also not found to be significantly greater than the $\log_{10}$ reductions obtained when an organic load was applied to the virus mixture and hard water was used in dilution. This statistical analysis is shown in Table 6.1.4.2.

Table 6.1.4.2: A Statistical Analysis of Log_{10} Reductions of Adenovirus Type 2 titer with Variable Conditions

Comparisons of Log_{10} Reductions of Adenovirus Type 2 Titer with Variable Conditions		
	***P(Pure > Hard)** A2; 1 min	***P(No Organic Load > Org Load)** A2; 1 min; Hard Water
Comparison of p-values for each disinfectant per category	**2/6 for P(Pure) 0 indet ****1 Disinfectant Excluded: #6*	7/12 for P(No Organic Load) 5 indet *3 Disinfectants Excluded: #1,2,3*
Overall Category p-value	p=0.891	p=0.387

**Probability that the Log_{10} Reductions of Viral Titer for X > the Log_{10} Reductions of Viral Titer for Z P(X>Z)*
***X/Y = # of CI's> 50% in each category/ Total # of CI's*
****Only disinfectant p-values with at least 5 determinant comparisons were used. Disinfectants with less than 5 determinant comparisons were excluded because the risk of false negatives is high when relatively few pairs of results are included in the analysis.*

6.2 Adenovirus Type 8

The study data from experiments involving adenovirus 8 are presented in Table 6.2.1. Results are expressed as the log_{10} reduction in adenovirus type 8 titer and represent the mean of multiple independent experimental trials. The complete tables showing all experimental values obtained with both 1- and 5- minute contact times are included in the Appendix as Tables 9.3 and 9.4. A statistical analysis of this germicide efficacy data is shown in Table 6.2.2.

Table 6.2.1: Effectiveness of Germicide Products Against Adenovirus Type 8 (with Hard Water)

Adenovirus Type 8 Disinfection Data (with hard water)		
Germicide	1 Minute Contact Time Results	5 Minute Contact Time Results
1) Steris Sterilant 20	2.6	4.7
2)Cidex-OPA	3.8	ND
3) Cidex	Complete Reduction ≥4.2	ND
4) Wavicide-01	Complete Reduction ≥4.2	ND
5) Clorox (1:50)	1.5	3.6
6) Clorox (1:10)	Complete Reduction ≥4.2	ND
7) Clorox Clean-up	4.0	ND
8)Vesphene Iise	0.29	0.68
9) 70% isopropyl alcohol	0.97	0.95
10) 70% Ethanol	4.0	4.2
11) Hydrogen Peroxide (3%)	0.31	1.1
12) Clorox Disinfecting Spray	Complete Reduction ≥4.2	Complete Reduction ≥4.3
13) Lysol Disinfecting Spray	3.4	4.2
14) TBQ	0.39	0.28
15) Povidone Iodine (10%)	0.88	0.80
16) Dettol (1:20)	0.55	0.37
17) Dettol (1:40)	0.40	0.30
18) CHG 4%	0.92	1.1
19) Medicated Soft & Sure	0.15	0.17
20) Acute-Kare	0.31	0.20
*1 minute: Viral Titer Mean = $10^{4.67}$ Viral Carrier Quantitation Mean = $10^{4.33}$ Mean of 7 Trials * All values are Log_{10} Viral Titer Reductions		*5 minute: Viral Titer Mean = $10^{4.64}$ Viral Carrier Quantitation Mean = $10^{4.33}$ Mean of 6 Trials

Formulations that demonstrated statistical significance in providing at least a 3 log_{10} reduction in adenovirus type 8 titer at both the 1 minute and 5 minute contact times, and therefore classified as "effective" germicides in the elimination of adenovirus type 8 from on environmental surfaces/inanimate objects included: Cidex-OPA (aldehyde, p= 0.008); Cidex (glutaraldehyde, p= 0.008); Wavicide-01 (glutaraldehyde, p= 0.008); Clorox 1:10 (halogen, p= 0.008); 70% Ethanol (alcohol, p= 0.008); and Clorox Disinfecting Spray (alcohol with QAC, p= 0.008). Clorox Clean-up (halogen, p= 0.062) and Lysol Disinfecting Spray (alcohol

with QAC, $p_{1\ min=}$ 0.062, $p_{5\ min=}$ 0.016) did not provide a statistically significant $\log_{10}$ reduction with a 1 minute contact time, but the statistical analysis suggests the possibility of an "effective" reduction because of the magnitude of the reductions observed and the relatively low but not quite significant P-values. Steris Sterilant 20 (acid) was found to be ineffective when a 1 minute contact time was used, giving only a 2.3 $\log_{10}$ reduction in adenovirus type 8 titer. The ineffectiveness of Steris, a high level disinfectant/chemical sterilant, can most likely be explained by poor virucidal activity when used with a 1 minute contact time at room temperature (approximately 20°C), instead of its recommended use-temperature (56°C).

With a 5 minute contact time, Steris Sterilant 20 (acid) and Clorox 1:50 (halogen) exhibited at least a 3 $\log_{10}$ reduction in adenovirus type 8 titer, yet this reduction was not statistically significant according to the established criteria. At a 1 minute contact time, less than a 3 $\log_{10}$ reduction in viral titer was observed. If allowed to dry for at least 5 minutes, Steris Sterilant 20 and Clorox 1:50 can be classified as effective against adenovirus type 8 because at least a 3-$\log_{10}$ reduction was achieved.

The following germicides were unable to provide a 3 $\log_{10}$ reduction of adenovirus type 8 titer after either a 1- or 5-minute contact time. Therefore, these germicides would be considered "ineffective" against adenovirus type 8: Vesphene IIse (phenol); 70% isopropyl alcohol (alcohol); 3% Hydrogen Peroxide (peroxide); TBQ (QAC); 10% Povidone Iodine (halogen); Dettol 1:20 (pine oil with alcohol); and Dettol 1:40 (pine oil with alcohol); CHG 4% (handwash); Medicated Soft and Sure (handwash); and Acute-Kare (alcohol).

Table 6.2.2: Statistical Analysis of Germicide Efficacy versus Adenovirus Type 8

Statistical Analysis Germicide Efficacy versus Adenovirus Type 8*		
	Adenovirus Type 8	
Disinfectant	**1 Minute Contact Time** (Hard Water)	**5 Minute Contact Time** (Hard Water)
1)Steris Sterilant 20	4/7 p-value =0.500 CI =50%	2/2 p-value =0.250 CI =75%
2)Cidex-OPA	**7/7** **p-value =0.008** **CI =99.2%**	No Data
3)Cidex	**7/7** **p-value =0.008** **CI =99.2%**	No Data
4)Wavicide-01	**7/7** **p-value =0.008** **CI =99.2%**	No Data
5)Clorox 1:50	1/7 p-value =0.992 CI =0.8%	5/6 p-value =0.109 CI =89.1%
6)Clorox 1:10	**7/7** **p-value =0.008** **CI =99.2%**	No Data
7)Clorox Clean-up	6/7 p-value =0.062 CI =93.8%	No Data
8)Vesphene IIse	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
9)70% isopropyl alcohol	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
10)70% ethanol	**7/7** **p-value =0.008** **CI =99.2%**	**6/6** **p-value =0.016** **CI =98.4%**
11)3% hydrogen peroxide	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
12)Clorox disinfecting spray	**7/7** **p-value =0.008** **CI =99.2%**	**6/6** **p-value =0.016** **CI =98.4%**
13)Lysol disinfecting spray	6/7 p-value =0.062 CI =93.8%	**6/6** **p-value =0.016** **CI =98.4%**
14)TBQ	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
15)Povidone-iodine	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
16)Dettol 1:20	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
17)Dettol 1:40	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
18)4% CHG	1/7 p-value =0.992 CI =0.8%	1/6 p-value =0.984 CI =1.6%
19)Medicated Soft and Sure	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
20)Acute Kare	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%

*Probability of a germicide providing at least a 3 $\log_{10}$ reduction of viral titer, being "effective"
***X /Y = # trials providing at least a 3.0 log reduction/ total # of trials*
Bold= effective disinfectants

Three out of four high-level disinfectants were effective in the inactivation of adenovirus type 8 and statistically significant (Cidex OPA, Cidex, Wavicide-01). The high-level disinfectant not effective with a 1-minute contact time, proved effective, although not statistically significant when a 5-minute contact time was used (Steris Sterilant 20). There were nine intermediate-level disinfectants tested for their effectiveness in reducing titers of adenovirus type 8. Of these nine intermediate-level disinfectants, five proved effective (Clorox 1:10, Clorox Clean-up, 70% ethanol, Clorox Disinfecting Spray, and Lysol Disinfecting Spray) and three were ineffective (Vesphene IIse, 3% Hydrogen Peroxide, and TBQ), regardless of the contact time, and one was found effective only at a 5 minute contact time(Clorox 1:50), but this effectiveness was not statistically significant. Among low-level disinfectants/antiseptics (n=6: 10% Povidone-iodine, Dettol 1:20, Dettol 1:40, 4% CHG, Medicated Soft and Sure, and Acute Kare), none were effective in reducing adenoviral titers with a 1-minute or 5-minute contact time.

When analyzed by type of active ingredient and using a 5-minute contact time, the following germicide types were capable of achieving a 3 $\log_{10}$ reduction in adenovirus 8 titer: peracetic acid; aldehyde; chlorine-based halogen; alcohol; and alcohol with QAC. The categories of disinfectant types that were not effective in eliminating adenovirus type 8 from environmental surfaces were: phenol; alcohol; peroxide; QAC; iodophor (halogen); pine oil with alcohol; and handwashes.

6.3 Adenovirus Type 2 versus Adenovirus Type 8

A comparison was made that analyzed the efficacy of germicides in the reduction of adenovirus type 2 versus adenovirus type 8. The data are shown in Tables 6.3.1 and 6.3.2.

In most cases, the germicide test products produced larger $\log_{10}$ reductions in adenovirus type 2 titer than adenovirus type 8 titer. But, these larger $\log_{10}$ reductions by adenovirus type 2 may be deceiving since the initial viral titer of adenovirus type 2 was larger than the initial viral titer of adenovirus type 8. Therefore, it may be more relevant to only compare the $\log_{10}$ reductions of the individual germicide products in the trials where the detection limits were not meet by both viruses. In these cases, the $\log_{10}$ reductions of adenovirus type 2 were greater for 10 of 13 comparable germicide products. However, when analyzed for statistical significance the $\log_{10}$ reductions of adenovirus type 2 titer were not significantly greater than the $\log_{10}$ reductions of adenovirus type 8 titer (Appendix 9.9).

Table 6.3.1: A Comparison of Germicide Efficacy Against Adenovirus Type 2 and versus Adenovirus Type 8, 1-Minute Contact Time

Comparison of Germicide Efficacy versus Adenovirus Strains (with Hard Water)		
Germicide	**1 Minute Contact Time Results**	
	Adenovirus Type 2	**Adenovirus Type 8**
1) Steris Sterilant 20	Complete Reduction ≥5.2	2.6
2)Cidex-OPA	Complete Reduction ≥5.2	3.8
3) Cidex	5.0	Complete Reduction ≥4.2
4) Wavicide-01	Complete Reduction ≥5.2	Complete Reduction ≥4.2
5) Clorox (1:50)	1.9	1.5
6) Clorox (1:10)	4.4	Complete Reduction ≥4.2
7) Clorox Clean-up	Complete Reduction ≥5.2	4.0
8)Vesphene IIse	1.2	0.29
9) 70% isopropyl alcohol	2.7	0.97
10) 70% Ethanol	4.2	4.0
11) Hydrogen Peroxide (3%)	0.51	0.31
12) Clorox Disinfecting Spray	4.4	Complete Reduction ≥4.2
13) Lysol Disinfecting Spray	2.8	3.4
14) TBQ	0.64	0.39
15) Povidone Iodine (10%)	1.3	0.88
16) Dettol (1:20)	0.41	0.55
17) Dettol (1:40)	0.45	0.40
18) CHG 4%	0.47	0.92
19) Medicated Soft & Sure	1.1	0.15
20) Acute-Kare	0.35	0.31
	*Viral Titer Mean = $10^{-8.42}$ *Viral Carrier Quantitation Mean = $10^{-5.49}$ *Mean of 5 Trials *Data Repeated from Table 5.1.1.1	* Viral Titer Mean = $10^{-8.67}$ * Viral Carrier Quantitation Mean = $10^{-4.23}$ *Mean of 7 Trials *Data Repeated from Table 5.2.1
	* All values are Log_{10} Viral Titer Reductions	

Table 6.3.2: A Comparison of Disinfectant Efficacy Against Adenovirus Type 2 and versus Adenovirus Type 8, 5 Minute Contact Time

Comparison of Germicide Efficacy versus Adenovirus Strains (with Hard Water)		
Disinfectant	**5 Minute Contact Time Results**	
	Adenovirus Type 2	**Adenovirus Type 8**
1) Steris Sterilant 20	Complete Reduction ≥6.3	4.7
2)Cidex-OPA	ND	ND
3) Cidex	ND	ND
4) Wavicide-01	ND	ND
5) Clorox (1:50)	Complete Reduction ≥6.3	3.67
6) Clorox (1:10)	5.15	ND
7) Clorox Clean-up	ND	ND
8)Vesphene IIse	4.3	0.68
9) 70% isopropyl alcohol	3.6	0.95
10) 70% Ethanol	4.8	4.2
11) Hydrogen Peroxide (3%)	0.95	1.1
12) Clorox Disinfecting Spray	4.5	Complete Reduction ≥4.3
13) Lysol Disinfecting Spray	4.5	4.2
14) TBQ	1.3	0.28
15) Povidone Iodine (10%)	1.2	0.80
16) Dettol (1:20)	1.8	0.37
17) Dettol (1:40)	1.2	0.30
18) CHG 4%	0.79	1.13
19) Medicated Soft & Sure	4.3	0.17
20) Acute-Kare	0.72	0.20
	*Viral Titer Mean = $10^{7.80}$ *Viral Carrier Quantitation Mean = $10^{5.49}$ *Mean of 5 Trials *Data Repeated from Table 5.1.1.1	* Viral Titer Mean = $10^{4.67}$ * Viral Carrier Quantitation Mean = $10^{4.23}$ *Mean of 6 Trials *Data Repeated from Table 5.2.1
	* All values are Log_{10} Viral Titer Reductions	

7. DISCUSSION

Surface disinfection as an infection control technique is quite controversial. This controversy exists because of the uncertainty associated with the exact role environmental surfaces play in the spread of infection. Some healthcare professionals feel that the use of disinfectants on environmental surfaces is excessive and unnecessary. They argue that there is no single study in the literature proving that routine environmental disinfection has a positive or beneficial effect on hospital-acquired infection rates (2-4, 17, 36). In addition, the argument is made that the use of surface disinfectants in healthcare facilities can cause occupational diseases such as skin irritation and allergies in housekeeping staff (2, 16, 45, 46). These claims are disputed by Rutala and Weber, and an eight-point argument in support of environmental disinfection has been offered (53). The specifics of this argument can be viewed in Section 3.3.3. Rutala and Weber feel that, "while noncritical surfaces are not commonly associated with transmission of infections to patients, one should clean and disinfect surfaces on a regular basis (53)." These sentiments are echoed in many of the published infection control handbooks and in professional recommendations for the control of infectious microbes found on noncritical surfaces in healthcare facilities (22, 24, 50, 51, 62, 64, 67).

Noncritical patient-care items are those which pose little or no threat of spreading infectious agents to patients. These types of items come into contact only with intact skin, but not mucous membranes (55). Inanimate environmental surfaces are considered

noncritical items since they too typically only come into contact with intact skin, and skin is considered to be a natural barrier to infectious agents. These surfaces are not generally viewed as important in the direct transmission of infectious agents, but, it is their role as a potential cross-contaminant that dictates their importance in infection control. Cross-contamination can result from "acquisition of transient hand carriage by healthcare professionals due to contact with a contaminated surface, or by patient contact with contaminated surfaces or medical equipment (53, 61)." Investigators have demonstrated that environmental surfaces near infected patients commonly become contaminated by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), which can survive for hours or days on dry surfaces (8, 27, 53, 56). Viruses, such as respiratory syncytial virus (RSV), adenovirus, and rotavirus, can also be isolated from dry environmental surfaces (59-61). Healthcare workers and patients that contact these surfaces can then potentially spread these infectious agents by their contaminated hands and medical equipment (53, 61). Therefore, these surfaces may contribute to endemic or epidemic spread of infection (27, 53).

The emergence of Severe Acute Respiratory Syndrome (SARS) in late 2002 (6) served as a strong stimulus for infection control professionals to re-examine their infection control procedures in healthcare facilities. One property that made SARS so infamous was its ability to cause hospital outbreaks. These outbreaks affected over 100 people, including hospital staff, patients, and visitors (6, 18). The transmission of SARS appeared to be primarily by respiratory droplets over short distances (6, 18). In rare cases though, SARS seemed to be spread by direct and indirect contact with respiratory secretions, feces, or animal vectors (6, 31, 40, 66, 82). More comprehensive studies on the SARS-Coronavirus

(CoV) found it to be more stable at room temperature than other human coronaviruses (6, 63). Other studies found that SARS-CoV could survive for up to forty-eight hours on plastic surfaces and up to four days in diarrhea (6, 47, 81). Nevertheless, disinfectants were found to effectively eliminate SARS-CoV infectivity (6, 47, 81). This evolving knowledge about SARS and its possible acquisition from hospital environmental surfaces led infection control professionals to refocus some of their infection control efforts on surface disinfection.

As the importance of surface disinfection in healthcare facility infection control programs has begun to be re-examined, so to has the literature dealing with the efficacy of germicide products against viral agents dried onto environmental surfaces. Two of the challenges facing infection control professionals when dealing with disinfection of environmental surfaces/inanimate objects are the identification of potential causative infectious agents and the matching of the most efficacious disinfection solutions versus the target pathogens to be eradicated from these items. Previous studies investigating the surface disinfection of viruses have been performed by Rutala et al. (54) and Sattar et al. (60). Rutala and colleagues performed a surface disinfection study on poliovirus and found that Clorox 1:10 and Lysol Disinfectant Spray were the germicide products with the greatest efficacy in reducing viral titer. In the study of Sattar and colleagues, the efficacy of a variety of germicide types versus coxackievirus B3, human parainfluenza virus type 3, human coronavirus 229E, and adenovirus type 5 dried on environmental surfaces was investigated. Sattar found that coxackievirus B3 was best inactivated by 0.5% and 1% sodium hypochlorite, 0.3% and 0.5% chloramines T, 2% glutaraldehyde, 0.04% QAC with 7% Hydrochloric acid (HCl), and 0.5% phenolic with 0.6% SDS. Sodium hypochlorite, chloramine T, 10% povidone-iodine, 70% ethanol, 2% glutaraldehyde, QAC in combination

with other disinfectants, and phenolics were all able to inactivate human parainfluenzavirus type 3 and human coronavirus 229E. Finally, adenovirus type 5 was inactivated by high concentrations of sodium hypochlorite and chloramine T, 70% ethanol, 2% glutaraldehyde, QAC in combination with other disinfectants, and phenolic combinations. Studies such as these serve as the basis for healthcare environmental surface disinfection standards that are created by infection control professionals.

The present study was performed to determine the effectiveness of a variety of germicide products in the inactivation of adenoviruses dried onto inanimate objects. Adenoviruses are frequent causes of respiratory illnesses which commonly manifest in infected individuals as respiratory tract infections and conjunctivitis. Adenoviral infections account for 5% to 8% of all pediatric respiratory illnesses (57) and occur worldwide in epidemic, endemic, and sporadic fashion. Adenovirus type 2 is both a respiratory and an enteric pathogen. It can be spread by direct contact with respiratory droplets or feces (57). Self-inoculation from contaminated fingers is the most important route of transmission and is known to occur after contact with contaminated environmental surfaces.

In contrast to adenovirus type 2, adenovirus type 8 typically causes eye infections such as keratoconjunctivitis and pharyngoconjunctivial fever (57). Nosocomial infections of the eye are generally viewed as uncommon, but, in reality, epidemic keratoconjunctivitis is a worldwide problem in newborns and remains a significant source of morbidity and mortality in those patients having cataract extraction or corneal replacement procedures (75). Hospital-acquired eye infections occur at a median rate of 0.24 infections per 10,000 discharges and overall represent around 0.5% of all nosocomial infections (44, 75). Epidemic keratoconjunctivitis (EKC) is the adenoviral illness most common in healthcare

settings (75). Transmission of adenovirus 8 can occur via the hands of medical personnel and by contaminated ophthalmic solutions and instruments such as tonometers and slit lamps (57, 75). Adenovirus 8 is extremely hardy when deposited on inanimate objects and fomites play an important role in its transmission (75). To prevent the spread of adenovirus 8, the CDC (11, 68) and the Association of Professionals in Infection Control and Epidemiology (50) recommend that tonometer tips be cleaned with soap and water, then disinfected, by soaking for 5 to 10 minutes in a solution containing either 5000 ppm chlorine, 3% hydrogen peroxide, 70% ethyl alcohol, or 70% isopropyl alcohol. Even though these recommendations exist, there are only limited data available on the efficacy of these disinfectant products versus adenovirus 8 (75). This deficiency in knowledge about the efficacy of modern surface disinfectants was one of the driving forces behind this study.

The protocol used in this experimental study simulates the drying of a viral agent on an inanimate object. Then, by treating the surface with a germicide test product, the efficacy of various germicides can be determined. The $\log_{10}$ reduction of the titer of the test organism is used as the measure of the germicide's efficacy. On the basis of this reduction, a comparison can be made of the efficacy of the various germicide product types based on active ingredients and formulation. The procedure used for these experiments was developed by SA Sattar (58). The only adaptations made to the Sattar protocol by our experimental group were: 1) changing the inoculum air drying time from 50 min to 40 min; 2) substituting glass vials for Teflon vials; and 3) transferring the stainless steel discs with disinfectant, after the desired contact time, into test tubes containing neutralizers, rather than placing the neutralizer onto the disc which is covered by disinfectant.

The results from this germicide efficacy study demonstrated that a number of products were effective at eliminating adenovirus type 2 dried onto inanimate objects at both a 1 minute and 5 minute contact time. Those germicides included Steris Sterilant 20; Cidex-OPA; Cidex; Wavicide-01; Clorox Clean-up; 70% Isopropyl Alcohol; 70% Ethanol; and Clorox Disinfecting Spray.

Results from two additional experiments gave further insight into germicide product efficacy. One further experiment showed that the hardness of the water used in dilution had little effect on germicide product efficacy in the inactivation of adenovirus type 2. When pure (sterile) water was used in germicide dilution instead of hard water (380-420 ppm as $CaCO_3$), the impact on the $\log_{10}$ reductions in viral titer differed only by an average of 0.62 $\log_{10}$ greater for pure water. Additionally, in no instance did the classification of the effectiveness of a germicide change because of the use of pure water instead of hard water. These findings imply that although the quality and disinfectant (chlorine) residual can vary in tap water at different locations, as well as at different times at the same location, ultimately this makes no difference in the virucidal activity of a germicide. This is important because it adds to the broad applicability of these results throughout all healthcare facilities.

Another variable condition of a set of experiments used adenovirus type 2 suspended in 5% FCS and germicide dilutions with hard water. This medium was used in order to investigate how the addition of protein or organic load in the form of 5% FCS might protect adenovirus type 2 from disinfection or change the virucidal properties of the germicide. These conditions more realistically model how adenoviruses are commonly found in healthcare facilities, i.e., encompassed in saliva or mucous. The results from these experiments demonstrated that the addition of an organic load reduced the effectiveness of

germicide products in the eradication of adenovirus type 2. The significance of these conditions for the log reductions of viral titer will be discussed below.

Because a wide range of germicide products were demonstrated to be effective in eliminating or substantially reducing adenovirus type 2 from test environmental surfaces, ICPs have many effective choices in deciding what products may be best suited for use in their healthcare facilities. ICPs can base their selection of appropriate germicides on disinfection class or active ingredient type. Before making this selection though, an infection control professional should analyze the available literature, manufacturer's recommendations, FDA recommendations, and infection control handbooks.

Only six of twenty germicide test products were found to be effective in the inactivation of adenovirus type 8 from our test environmental surfaces. The germicide test products found to be effective at both a 1 and 5 minute contact time were Cidex-OPA; Cidex; Wavicide-01; Clorox 1:10; 70% Ethanol; and Clorox Disinfecting Spray. Thus, effective germicide choices for the eliminaton of adenovirus type 8 are more limited than those available to adenovirus type 2 inactivation. Clorox Clean-up and Lysol Disinfecting Spray were unable to provide a statistically significant 3.0 log reduction of adenoviral titer, but the magnitude and consistency of their log reductions and nearly significant P-values ($0.10 > p\text{-value} > 0.05$) still suggests that these products may still be effective.

The $\log_{10}$ reductions provided by the germicide products can be compared over the various experimental conditions to determine if any trends exist. These comparisons are shown in Table 7.1 and a complete version is available in the Appendix (Appendix Table 9.9).

Table 7.1: Comparison of Log_{10} Reductions for Germicide Products for the Different Experimental Variables of Contact Time, Test Water, and Adenovirus Type

Comparisons of Adenovirus Log_{10} Reductions with Variable Conditions					
	*P(A2-5min > A2-1min)	*P(A8-5min > A8-1 min)	*P(A2 > A8) 1 min	*P(Pure > Hard) A2; 1 min	*P(No Organic Load > Org Load) A2; 1 min; Hard Water
Overall Category p-value	P=0.006	p= 0.709	P=0.402	p=0.891	p=0.387
*P(X>Z)= *Probability that the Log_{10} Reductions of Viral Titer for X > the Log_{10} Reductions of Viral Titer for Z*					

From this analysis it is possible to conclude that the log_{10} reductions of adenovirus type 2 titer are greater when a 5 minute contact time is used than a 1 minute contact time. This is in contrast to the log_{10} reductions of adenovirus type 8, which are not significantly greater when a 5 minute contact time is used versus a 1 minute contact time. When the experimental variable condition of water type is examined it is found that the log_{10} reductions of adenovirus type 2 are not significantly greater when pure water is used in dilution instead of hard water, and the log_{10} reductions of adenovirus type 2 are not significantly greater when the virus is pure (clarified, chloroform-extracted cell culture lysate) as opposed to addition of organic load (5% fetal calf serum). Finally, we can conclude that the log_{10} reductions of adenovirus type 2 by germicide products are not significantly greater than the log_{10} reductions of adenovirus type 8.

As mentioned previously, to prevent the spread of adenovirus 8 in the ophthalmic setting, the CDC (11, 68) and the Association of Professionals in Infection Control and Epidemiology [APIC] (50) have previously recommended that tonometer tips be cleaned with soap and water, then disinfected by soaking for 5 to 10 minutes in a solution containing either 5000 ppm chlorine, 3% hydrogen peroxide, 70% ethyl alcohol, or 70% isopropyl alcohol. Although these recommendations have existed for some years, there are only

limited data available to support them. An interesting finding from our study was that, of the four disinfectants recommended by the CDC and APIC for elimination of adenovirus type 8 from the environment, two (70% isopropyl alcohol and 3% hydrogen peroxide) were found to be relatively ineffective. In addition, it is possible a third CDC recommended disinfectant is ineffective. This uncertainty exists because there is confusion as to what dilution of chlorine was actually intended by the author in the original CDC recommendation (68), 5000 ppm chlorine or 500 ppm chlorine. The original recommendations call for 5000 ppm chlorine (68), but other publications cite this original CDC recommendation and state the value as 500 ppm chlorine(11, 50). The author also has affirmed that the 500 ppm value was what he actually intended (Dr. William Rutala, personal communication). Examining these two values closely, we find that 5000 ppm chlorine, comparable to the Clorox 1:10 (6000ppm) is an "effective" disinfectant, but that 500 ppm chlorine is "ineffective", when compared to the germicide efficacy study results for Clorox 1:50 (1200 ppm). This observation implies that the current disinfection recommendations followed by infection control professionals to prevent human exposure to adenovirus type 8 need to be re-examined and revised.

When choosing an appropriate germicide for treating items contaminated with adenovirus type 8, many factors such as effectiveness, cost, ease of preparation, application, and safety must be taken into consideration. When considering these factors, an infection control professional must decide which are of the greatest importance in their healthcare facility; nevertheless, effectiveness must usually be considered first. If cost is found to be important, chlorine, iodophors, phenolics, or quaternary ammonium compounds are the disinfectant types which have the lowest cost/gallon at use-dilution according to Table

3.6.1.1. When examining ease of preparation, some germicides must be diluted or activated before use, while others are able to be used as purchased from the manufacturer. Of the germicide products we tested, all were liquids and would be sprayed onto environmental surfaces. Finally, safety of the infection control professional when applying the germicide, and potential contamination of the environment upon disposal of the germicide are factors for consideration. As the disinfectant class increases from low-level to intermediate-level to high-level disinfectants, the safety risk also potentially increases.

With these variables in mind, germicide recommendations can be made for the elimination of adenovirus type 8. This study found that Cidex OPA, Cidex, Wavicide-01, Clorox 1:10 and 70% ethanol, which are all high-level disinfectants or intermediate-level disinfectants currently recommended by the CDC, are the best candidate virucides. These products were chosen because a high-level disinfectant must be used to disinfect semicritical surfaces, i.e., applanation tonometers. Other products that are alcohol or chlorine-based (Lysol Disinfecting Spray; Clorox Disinfecting Spray; and Clorox Clean-up) may be suitable germicide choices as well, but were not recommended because they have not been FDA-approved as high-level disinfectants or previously recommended for use by the CDC. Another consideration when choosing one of the recommended germicides is the effective wash-off of these germicides from the tonometer tips. This is important because tonometers are used in the eyes, and germicide residue from some products, like aldehydes, can potentially cause adverse health outcomes. In addition, Clorox 1:10 has been found to damage tonometer tips when used in disinfection (12). This obviously is an undesired outcome, and is another factor that must be addressed in product selection. Finally, all the

recommended disinfectants should be allowed to contact all environmental surfaces for at least five minutes, ensuring that maximal reduction in adenovirus titers occur.

Because of its perceived stability on inanimate objects, adenovirus type 8 has the potential to be regarded as the supreme test for adenovirus disinfection. However, when the $\log_{10}$ reductions of adenovirus type 2 were compared with adenovirus type 8 no significant difference was observed. If a differential susceptibility exists for all other adenovirus serotypes and adenovirus type 8, then it is possible adenovirus type 8 could be considered the "gold standard" for adenovirus surface germicide efficacy testing. However comparisons made through controlled experimental trials would need to be done to confirm this hypothesis.

This study had several limitations which may contribute to some uncertainty about the conclusions. The first of these limitations deals with the stainless steel disc used to simulate environmental surfaces/inanimate objects. This stainless steel disc cannot perfectly mimic other environmental surfaces such as countertops and tonometers as they exist in healthcare settings. Therefore, the surface disinfectant test products or test viruses might react differently in the real-life healthcare environment than in the experimental setting. But, the main consideration in considering viral susceptibility remains, whether the virus is dry (carrier test) or wet (suspension test). It is our belief that as long as the virus is completely dry, its susceptibility to germicide products will remain constant regardless of the inanimate object on which it is found. The only other consideration would be the accessability of the virus to the disinfectant, which may be influenced by how smooth or how rough or porous the environmental surface is. This study examined the respone of adenoviruses 2 and 8 to germicides only on a hard, relatively smooth surface.

A second limitation of the study pertains to the fact that not all known adenovirus serotypes were tested to determine the susceptibility of each one to each germicide test product. Adenovirus type 2 and adenovirus type 8 were chosen as representative adenovirus strains. These strains were selected because adenovirus type 2 has been evaluated previously in the literature and adenovirus type 8 is generally considered a very hardy strain with unknown germicide susceptibility. Because all other serotypes were not tested, it is not known how they would interact with the test germicides when dried on inanimate objects nor is it known how vulnerable the various adenovirus serotypes are to disinfection as compared to adenovirus type 2 and 8. However, since there are 49 different human adenovirus serotypes, it would be logistically challenging to test the surface disinfectant susceptibility of all those strains.

Another limitation of this study is its rather narrow pathogen focus since adenovirus was the only pathogen tested. Accordingly, these test results can not be applied to other potential microbes present in hospitals, some of which may be more important causes of nosocomial infection. This study was designed to determine the most effective germicide for the elimination of adenovirus type 8 and this goal was accomplished. However, it cannot be assumed that other nosocomial viral pathogens will be equally susceptible to the test germicides.

Although twenty different disinfectant types were evaluated in this study, all available disinfectant products were not tested. A representative group of disinfectant types was chosen, but there are thousands of commercial and household disinfectants available and this makes it virtually impossible to test all existing products. Active ingredient was a determining factor for the representative disinfectant products that were chosen. This study

tested the following disinfectant types: acid; aldehyde; glutaraldehyde; halogen; phenol; alcohol; peroxide; quaternary ammonium compound (QAC); alcohol combined with QAC; pine oil combined with alcohol; biguanides, surfacants; and handwashes.

No absolute international standard of $\log_{10}$ reduction in viral titer exists to define germicide efficacy. This lack of an established gold standard can also be seen as a study limitation. Because of this limitation, an arbitrary standard of at least a 3 $\log_{10}$ reduction of viral titer was chosen as the level of effectiveness in order to make a valid comparison of germicide efficacy. This number was chosen based on recommendations in the literature from Sattar (58), Steinmann (65), and Rutala (54). In addition, that reduction in adenoviral titer is believed to be necessary to safely eliminate patient risk associated with tonometer use after disinfection.

The selection of the germicide "effectiveness" level led to the discovery of another study limitation. The selection of the germicide effectiveness level was based on published literature (54, 58, 65) and a general consensus about the necessary reduction in adenoviral titer believed to safely eliminate patient risk associated with tonometer use after disinfection. However, the actual level of adenovirus type 8 transferred from an infected patient to a tonometer is unknown. It has been estimated that 3-4 $\log_{10}$ of adenovirus type 8 are transferred from an infected patient to the tonometer (Dr. William Rutala, personal communication), but this estimate has not been verified through independent experimental trials. Without this verification, the potential impact of these results cannot fully be established.

Another limitation of this study is the limited amount of quality comparative data in the literature. Viral disinfection has been infrequently studied. When studies have been

conducted, a broad range of viruses have been the subject of investigation. Some of the most common viruses studied have been poliovirus, influenza virus, and adenovirus. Studies on the surface disinfection of adenovirus typically have focused on serotypes 2 and 5. Little is known about the disinfection of adenovirus type 8, other than its percieved hardiness, and adenovirus type 8 was the focus of this investigation. Therefore, any results obtained in this study have few comparative data to help establish reproducability or assess comparability.

A final limitation of this study lies in the variability of the test method used. As mentioned previously, there are two main ways to perform surface disinfection testing, carrier testing and suspension testing. Results from the suspension testing method are viewed as less accurate because that method simulates the actual healthcare environmental surface less realistically than the carrier test (61). The carrier test protocol developed by Sattar has recently become the accepted method (58). However, much of the literature available on environmental surface/inanimate object disinfection has been obtained using suspension testing, so the results from the existing literature need to be viewed and interpreted cautiously.

8. CONCLUSIONS

This germicide efficacy study evaluated how well candidate germicides reduced adenovirus type 2 and adenovirus type 8 infectivity from simulated inanimate objects in healthcare settings. Eight of the twenty disinfectant products tested were found to be effective in eliminating or appreciably reducing the infectivity of adenovirus type 2 dried onto environmental surfaces/inanimate objects and eight of twenty products were found to be effective in eliminating or appreciably reducing the infectivity of adenovirus type 8 from environmental surfaces/inanimate objects.

As would have generally been predicted, the higher the level of germicide class the more effective the germicide product was in eliminating or appreciably reducing the infectivity of the adenovirus strains. The level of effectiveness varied among germicide products based on active ingredient. Germicides whose active ingredients was peracetic acid, glutaraldehyde, aldehyde, chlorine-based halogen, alcohol, and alcohol mixed with QAC proved most effective in eliminating or reducing adenovirus infectivity.

An unanticipated finding was that the efficacy of the test germicide products was not significantly affected when the experimental variable of water quality for germicide preparation were varied (Appendix Table 9.9). The water quality variables examined were preparation of the germicides with hard water (380-420 ppm as $CaCl_2$) versus pure sterile water and the disinfection of adenovirus when surrounded by an organic load. When pure water was used instead of hard water, germicide efficacy was unaffected. When the viral

particles were in the presence of an organic load (modeled with 5% fetal calf serum), as is commonly found in actual healthcare settings, the effectiveness of the germicide was also not significantly affected.

Also unexpected was the finding that adenovirus type 8 was no more resistant to the germicides than was adenovirus type 2. Adenovirus type 8 is generally regarded as one of the hardiest of all adenovirus strains, and therefore was potentially viewed as the "gold test standard" for adenovirus surface disinfection testing. However, this status can not be considered true according to our experimental and statistical analyses.

The primary goal of this project was to determine what germicide types were most effective in the elimination or substantial reduction of adenovirus type 8 from inanimate objects. This was the focus of this study because this virus is associated with hospital outbreaks of epidemic keratoconjunctivitis (EKC) and there is limited information is available concerning the efficacy of surface disinfection products against it. Currently, the CDC (11, 68) and the Association of Professionals in Infection Control and Epidemiology [APIC] (50) recommend that tonometer tips be cleaned with soap and water, then disinfected, by soaking for 5 to 10 minutes in a solution containing either 5000 ppm chlorine, 3% hydrogen peroxide, 70% ethyl alcohol, or 70% isopropyl alcohol. However, this study found that 2 and possibly 3 of these recommended disinfection products, 3% hydrogen peroxide, 70% isopropyl alcohol, and 500 ppm chlorine (if this is the dilution intended by the author), were ineffective in eliminating or appreciably reducing adenovirus type 8. The literature also states that sometimes 70% isopropyl alcohol wipes are used in the disinfection of tonometer tips, because a short and simple disinfection procedure is desired (15). Craven suggests that this disinfection technique may prove effective in adenovirus elimination (15). However,

once again the results of our study dispute this notion as 70% isopropyl alcohol was found to be ineffective in eliminating or appreciably reducing adenovirus type 8. As a consequence, current healthcare infection control standards relating to adenoviruses should perhaps be reexamined and possibly rewritten.

In summary the efficacy of germicide products is dependent upon many factors. When selecting an appropriate adenovirus virucide for healthcare facilities, ICPs must determine what will work best given their individual set of circumstances. It is also important to understand that, just because a germicide test product is effective in the disinfection of adenovirus, this does not imply effectiveness versus other viral pathogens of nosocomial importance. If appropriately applied, these experimental findings provide disinfection options that can reduce a patient's risk of acquiring adenovirus type 8 from healthcare environmental surfaces, i.e., tonometer tips, and thus prevent a commonly occurring nosocomial eye infection. These findings also provide useful information for effective germicidal control of adenoviruses on other surfaces in healthcare settings.

9. APPENDICES

Appendix Table 9.1: Effectiveness of Germicide Products Against Adenovirus Type 2 with a 1 minute contact time, Complete Version

Germicide	Adenovirus Type 2 Disinfection Data (with Hard Water) 1 Minute Contact Time Results					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean
1) Steris Sterilant 20	Complete Reduction 5.67	4.83	Complete Reduction 5.3	Complete Reduction 6.0	Complete Reduction 4.0	Complete Reduction 5.16
2) Cidex-OPA	Complete Reduction 5.67	Complete Reduction 5.5	Complete Reduction 5.3	Complete Reduction 5.67	Complete Reduction 4.0	Complete Reduction 5.23
3) Cidex	Complete Redcution 5.67	Complete Reduction 5.5	Complete Reduction 5.3	Complete Reduction 5.67	3.33	5.09
4) Wavicide-01	Complete Reduction 5.67	Complete Reduction 5.5	Complete Reduction 5.3	Complete Reduction 5.67	Complete Reduction 4.0	Complete Reduction 5.23
5) Clorox (1:50)	3.67	1.83	1.3	2.17	1.0	1.99
6) Clorox (1:10)	Complete Reduction 5.67	1.5	Complete Reduction 5.3	Complete Reduction 5.67	Complete Reduction 4.0	4.43
7) Clorox Clean-up	Complete Reduction 5.67	Complete Reduction 5.5	Complete Reduction 5.3	Complete Reduction 5.67	Complete Reduction 4.0	Complete Reduction 5.23
8)Vesphene IIse	3.0	0.2	No Reduction	1.0	1.7	1.18
9) 70% isopropyl alcohol	4.0	3.5	2.8	3.0	No Reduction	2.66
10) 70% Ethanol	5.0	4.83	4.0	3.17	Complete Reduction 4.0	4.2
11) Hydrogen Peroxide (3%)	1.0	0.25	0.3	0.67	0.33	0.51
12) Clorox Disinfecting Spray	4.0	4.83	3.3	Complete Reduction 5.67	Complete Reduction 4.0	4.36
13) Lysol Disinfecting Spray	2.67	3.0	3.0	3.0	2.33	2.8
14) TBQ	0.37	0.5	No reduction	2.0	0.33	0.64
15) Povidone Iodine (10%)	1.17	0.83	1.3	1.0	2.33	1.33
16) Dettol (1:20)	0.37	0.5	No Reduction	1.17	No Reduction	0.41
17) Dettol (1:40)	0.17	No Reduction	No Reduction	0.37	1.7	0.45
18) CHG 4%	0.67	1.0	No Reduction	0.67	No Reduction	0.47
19) Medicated Soft & Sure	1.0	1.2	0.63	1.33	1.33	1.10
20) Acute-Kare	0.17	0.75	No Reduction	0.5	0.33	0.35
	*Trial 1 Viral Titer = $10^{8.3}$ Carrier Quantitation = $10^{5.67}$ *Trial 2 Viral Titer = $10^{8.67}$ Carrier Quantitation = $10^{5.5}$ *Trial 3 Viral Titer = $10^{8.3}$ Carrier Quantitation = $10^{5.3}$ *All values are Log_{10} Viral Titer Reductions			*Trial 4 Viral Titer = $10^{7.67 \text{ to } 8.3}$ Carrier Quantitation = $10^{5.67 \text{ to } 6.0}$ *Trial 5 Viral Titer Mean = $10^{8.3}$ Carrier Quantitation Mean = $10^{4.0}$ *Trial Mean Viral Titer = $10^{7.95}$ Carrier Quantitation = $10^{5.27}$		

Appendix Table 9.2: Effectiveness of Germicide Products Against Adenovirus Type 2 with a 5 minute contact time, Complete Version

Germicide	**Adenovirus Type 2 Disinfection Data** (with Hard Water) **5 Minute Contact Time Results**			
	Trial 1	Trial 2	Trial 3	Mean
1) Steris Sterilant 20	ND	ND	Complete Reduction 6.3	Complete Reduction 6.3
2) Cidex-OPA	Not Done (ND)	ND	ND	ND
3) Cidex	ND	ND	ND	ND
4) Wavicide-01	ND	ND	ND	ND
5) Clorox (1:50)	ND	ND	Complete Reduction 6.3	Complete Reduction 6.3
6) Clorox (1:10)	ND	Complete Reduction 5.67	4.63	5.15
7) Clorox Clean-up	ND	ND	ND	ND
8)Vesphene IIse	4.17	2.37	Complete Reduction 6.3	4.28
9) 70% isopropyl alcohol	3.0	3.67	4.0	3.56
10) 70% Ethanol	2.37	Complete Reduction 5.67	Complete Reduction 6.3	4.78
11) Hydrogen Peroxide (3%)	1.17	1.17	0.5	0.95
12) Clorox Disinfecting Spray	Complete Reduction 5.67	1.8	Complete Reduction 5.0	4.16
13) Lysol Disinfecting Spray	4.0	3.3	Complete Reduction 5.0	4.1
14) TBQ	1.37	1.37	1.3	1.35
15) Povidone Iodine (10%)	3.0	No Reduction	0.7	1.23
16) Dettol (1:20)	1.67	1.67	2.0	1.78
17) Dettol (1:40)	1.67	ND	0.8	1.24
18) CHG 4%	1.0	0.67	0.7	0.79
19) Medicated Soft & Sure	Complete Reduction 5.67	4.0	3.5	4.39
20) Acute-Kare	1.67	No Reduction	0.5	0.72
	*Trial 1 Viral Titer = $10^{-7.5}$ Carrier Quantitation = $10^{-5.67}$ *Trial 2 Viral Titer Mean = $10^{-6.99}$ Carrier Quantitation Mean = $10^{-4.49}$ *Trial 3 Viral Titer = $10^{-8.5}$ Carrier Quantitation = $10^{-6.3}$ *Trial Mean Viral Titer Mean = $10^{-7.66}$ Carrier Quantitation Mean = $10^{-5.49}$ *All values are Log_{10} Viral Titer Reductions			

Appendix Table 9.3: Effectiveness of Germicide Products Against Adenovirus Type 8 with a 1 minute contact time, Complete Version

Germicide	Adenovirus Type 8 Disinfection Data (with Hard Water) 1 Minute Contact Time Results							
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Mean
1) Steris Sterilant 20	Complete Reduction 3.0	Complete Reduction 3.5	0.17	3.3	2.0	3.7	2.83	2.64
2) Cidex-OPA	Complete Reduction 3.0	Complete Reduction 3.5	Complete Reduction 4.67	Complete Reduction 4.3	Complete Reduction 3.67	4.0	Complete Reduction 4.5	3.85
3) Cidex	Complete Reduction 3.0	Complete Reduction 3.5	Complete Reduction 4.67	Complete Reduction 4.3	Complete Reduction 3.67	Complete Reduction 6.0	Complete Reduction 4.5	Complete Reduction 4.23
4) Wavicide-01	Complete Reduction 3.0	Complete Reduction 3.5	Complete Reduction 4.67	Complete Reduction 4.3	Complete Reduction 3.67	Complete Reduction 6.0	Complete Reduction 4.5	Complete Reduction 4.23
5) Clorox (1:50)	No Reduction	2.0	0.67	2.8	1.0	4.0	0.5	1.57
6) Clorox (1:10)	Complete Reduction 3.0	Complete Reduction 3.5	Complete Reduction 4.67	Complete Reduction 4.3	Complete Reduction 3.67	Complete Reduction 6.0	Complete Reduction 4.5	Complete Reduction 4.23
7) Clorox Clean-up	Complete Reduction 3.0	Complete Reduction 3.5	Complete Reduction 4.67	2.63	Complete Reduction 3.67	Complete Reduction 6.0	Complete Reduction 4.5	4.00
8)Vesphene Iise	No Reduction	0.2	0.17	0.63	0.17	0.33	0.5	0.29
9) 70% isopropyl alcohol	0.33	1.5	0.67	0.8	1.17	1.33	1.0	0.97
10) 70% Ethanol	Complete Reduction 3.0	Complete Reduction 3.5	Complete Reduction 4.67	3.3	Complete Reduction 3.67	Complete Reduction 6.0	Complete Reduction 4.5	4.09
11) Hydrogen Peroxide (3%)	No Reduction	0.5	0.37	No Reduction	No Reduction	1.33	No Reduction	0.31
12) Clorox Disinfecting Sp	Complete Reduction 3.0	Complete Reduction 3.5	Complete Reduction 4.67	Complete Reduction 4.3	Complete Reduction 3.67	Complete Reduction 6.0	Complete Reduction 4.5	Complete Reduction 4.23
1. ol Disinfecting Spray	Complete Reduction 3.0	Complete Reduction 3.5	3.0	2.3	Complete Reduction 3.67	5.33	3.5	3.47
14) TBQ	No Reduction	0.83	0.17	No Reduction	0.37	1.33	No Reduction	0.39
15) Povidone Iodine (10%)	No Reduction	1.5	1.0	0.3	1.0	1.5	0.83	0.88
16) Dettol (1:20)	No Reduction	0.2	0.17	0.63	1.0	1.33	0.5	0.55
17) Dettol (1:40)	No Reduction	0.2	0.67	No Reduction	0.37	1.33	0.2	0.40
18) CHG 4%	0.33	Complete Reduction 3.5	2.0	0.3	No Reduction	0.33	No Reduction	0.92
19) Medicated Soft & Sure	No Reduction	0.2	No Reduction	No Reduction	0.17	0.7	No Reduction	0.15
20) Acute-Kare	No Reduction	0.5	No Reduction	0.3	0.17	0.7	0.5	0.31

*Trial 1
Viral Titer = $10^{7.0}$
Carrier Quantitation = $10^{3.0}$
*Trial 2
Viral Titer = $10^{<5.67}$
Carrier Quantitation = $10^{3.5}$
*Trial 3
Viral Titer = $10^{7.5}$
Carrier Quantitation = $10^{4.83}$
*Trial 4
Viral Titer = $10^{7.67}$
Carrier Quantitation = $10^{4.3}$
*Trial 5
Viral Titer Mean = $10^{7.5}$
Carrier Quantitation Mean = $10^{3.67}$
*Trial 6
Viral Titer Mean = ?
Carrier Quantitation Mean = $10^{5.0}$
*Trial 7
Viral Titer = $10^{6.83}$
Carrier Quantitation = $10^{4.5}$
*Trial Mean
Viral Titer = $10^{6.81}$
Carrier Quantitation = $10^{4.23}$
*All values are Log_{10} Viral Titer Reductions

Appendix Table 9.4: Effectiveness of Germicide Products Against Adenovirus Type 8 with a 5 minute contact time, Complete Version

Germicide	Adenovirus Type 8 Disinfection Data (with Hard Water) 5 Minute Contact Time Results						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Mean
1) Steris Sterilant 20	ND	ND	ND	ND	5.0	4.5	4.75
2) Cidex-OPA	ND	ND	ND	ND	ND	ND	ND
3) Cidex	ND	ND	ND	ND	ND	ND	ND
4) Wavicide-01	ND	ND	ND	ND	ND	ND	ND
5) Clorox (1:50)	Complete Reduction 4.3	Complete Reduction 3.67	4.5	1.7	4.33	3.5	3.67
6) Clorox (1:10)	ND	ND	ND	ND	ND	ND	ND
7) Clorox Clean-up	ND	ND	ND	ND	ND	ND	ND
8)Vesphene Iise	0.63	0.37	0.2	1.0	0.7	1.2	0.68
9) 70% isopropyl alcohol	1.0	2.0	No Reduction	0.7	0.5	1.5	0.95
10) 70% Ethanol	Complete Reduction 4.3	Complete Reduction 3.67	Complete Reduction 4.5	Complete Reduction 4.0	4.33	Complete Reduction 4.5	4.22
11) Hydrogen Peroxide (3%)	0.3	No Reduction	0.2	0.33	0.33	0.5	1.13
12) Clorox Disinfecting Spray	Complete Reduction 4.3	Complete Reduction 3.67	Complete Reduction 4.5	Complete Reduction 4.0	Complete Reduction 5.0	Complete Reduction 4.5	Complete Reduction 4.33
13) Lysol Disinfecting Spray	Complete Reduction 4.3	Complete Reduction 3.67	Complete Reduction 4.5	Complete Reduction 4.0	4.33	Complete Reduction 4.5	4.22
14) TBQ	No Reduction	No Reduction	No Reduction	0.33	0.33	1.0	0.28
15) Povidone Iodine (10%)	0.3	1.0	No Reduction	0.5	1.0	2.0	0.80
16) Dettol (1:20)	No Reduction	.17	No Reduction	0.7	0.5	0.83	0.37
17) Dettol (1:40)	No Reduction	No Reduction	0.2	0.5	0.33	0.75	0.30
18) CHG 4%	Complete Reduction 4.3	ND	0.2	0.33	0.33	0.5	1.13
19) Medicated Soft & Sure	No Reduction	No Reduction	No Reduction	0.5	No Reduction	0.5	0.17
20) Acute-Kare	No Reduction	No Reduction	No Reduction	0.7	No Reduction	0.5	0.20
	*Trial 1 Viral Titer = $10^{6.67}$ Carrier Quantitation = $10^{4.3}$ *Trial 2 Viral Titer = $10^{6.5}$ Carrier Quantitation = $10^{3.67}$ *Trial Mean Viral Titer = $10^{6.64}$ Carrier Quantitation = $10^{4.33}$ *All values are Log_{10} Viral Titer Reductions		*Trial 3 Viral Titer = $10^{6.67}$ Carrier Quantitation = $10^{4.5}$ *Trial 4 Viral Titer = $10^{6.67}$ Carrier Quantitation = $10^{4.0}$		*Trial 5 Viral Titer = $10^{7.3}$ Carrier Quantitation = $10^{5.0}$ *Trial 6 Viral Titer = $10^{6.0}$ Carrier Quantitation = $10^{4.5}$		

Appendix Table 9.5: A Comparison of the Recovery of Adenovirus Type 2 from Stainless Steel Carrier Discs When Neutralization Takes Place in a Glass Vial versus in a Glass Test Tube

Adenovirus Type 8 Stainless Steel Disc Carrier Quantitation Sub-experiment													
	Dilution (& # of wells showing CPE)												
Experimental variables	-1		-2		-3		-4		-5		-6		Viral Titer
	Day 9	13	9	13	9	13	9	13	9	13	9	13	
V_{40a}	4/4	4/4	1/4	4/4	0/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	$10^{-3.5}$
V_{40b}	4/4	4/4	1/4	4/4	0/4	4/4	0/4	2/4	0/4	0/4	0/4	0/4	10^{-4}
V_{40c}	4/4	4/4	1/4	4/4	0/4	4/4	0/4	1/4	0/4	0/4	0/4	0/4	$10^{-3.67}$
V_{40d}	4/4	4/4	1/4	4/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4	10^{-3}
V_{40} Carrier Quantitation Mean													**$10^{-3.54}$**
TT_{40a}	4/4	4/4	0/4	4/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4	10^{-3}
TT_{40b}	4/4	4/4	0/4	4/4	0/4	3/4	0/4	1/4	0/4	0/4	0/4	0/4	$10^{-3.67}$
TT_{40c}	4/4	4/4	0/4	4/4	0/4	4/4	0/4	2/4	0/4	0/4	0/4	0/4	10^{-4}
TT_{40d}	4/4	4/4	0/4	4/4	0/4	4/4	0/4	1/4	0/4	0/4	0/4	0/4	$10^{-3.67}$
TT_{40} Carrier Quantitation Mean													**$10^{-3.59}$**
V_{70a}	4/4	4/4	0/4	4/4	0/4	4/4	0/4	1/4	0/4	0/4	0/4	0/4	$10^{-3.67}$
V_{70b}	4/4	4/4	1/4	4/4	0/4	3/4	0/4	0/4	0/4	0/4	0/4	0/4	$10^{-3.5}$
V_{70c}	4/4	4/4	0/4	4/4	0/4	3/4	0/4	0/4	0/4	0/4	0/4	0/4	$10^{-3.5}$
V_{70d}	4/4	4/4	0/4	4/4	0/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	$10^{-3.5}$
V_{70} Carrier Quantitation Mean													**$10^{-3.44}$**
TT_{70a}	4/4	4/4	0/4	4/4	0/4	3/4	0/4	1/4	0/4	0/4	0/4	0/4	$10^{-3.67}$
TT_{70b}	4/4	4/4	1/4	4/4	0/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	$10^{-3.5}$
TT_{70c}	4/4	4/4	1/4	4/4	0/4	3/4	0/4	0/4	0/4	0/4	0/4	0/4	$10^{-3.5}$
TT_{70d}	4/4	4/4	0/4	4/4	0/4	4/4	0/4	2/4	0/4	0/4	0/4	0/4	10^{-4}
TT_{70} Carrier Quantitation Mean													**$10^{-3.61}$**
V: Glass vial 40: 40 minute Contact time *X/Y= # of cells with CPE/ # of total cells											TT: Test tube 70: 70 minute contact time		

Adenovirus Type 8 Quantitation										
-4		-5		-6		-7		-8		
9	*13*	*9*	*13*	*9*	*13*	*9*	*13*	*9*	*13*	Day
1/4	4/4	0/4	2/4	0/4	1/4	0/4	0/4	0/4	0/4	

*Viral Titer: $10^{-5.67}$

Appendix Table 9.6: A Statistical Analysis of the Germicide Efficacy Data

Germicide Efficacy Analysis

(Probability of a germicide providing at least a 3 $\log_{10}$ reduction of viral titer, being "effective")

	Adenovirus Type 2				Adenovirus Type 8	
Disinfectant	**1 Minute Contact Time** (Hard Water)	**5 Minute Contact Time** (Hard Water)	**Pure Water in Dilution** (1 min CT)	**Organic Load with Hard Water** (1 min CT)	**1 Minute Contact Time** (Hard Water)	**5 Minute Contact Time** (Hard Water)
1)Steris Sterilant 20	**5/5 p-value = 0.031 ***CI =96.9%	1/1 p-value =0.500 CI =50%	2/2 p-value =0.250 CI =75%	2/2 p-value =0.250 CI =75%	4/7 p-value =0.500 CI =50%	2/2 p-value =0.250 CI =75%
2)Cidex-OPA	5/5 p-value =0.031 CI =96.9%	No Data	Pure water not used in dilution	2/2 p-value =0.250 CI =75%	7/7 p-value =0.008 CI =99.2%	No Data
3)Cidex	5/5 p-value =0.031 CI =96.9%	No Data	Pure water not used in dilution	2/2 p-value =0.250 CI =75%	7/7 p-value =0.008 CI =99.2%	No Data
4)Wavicide-01	5/5 p-value =0.031 CI =96.9%	No Data	Pure water not used in dilution	2/2 p-value =0.250 CI =75%	7/7 p-value =0.008 CI =99.2%	No Data
5)Clorox 1:50	1/5 p-value =0.969 CI =3.1%	1/1 p-value =0.500 CI =50%	½ p-value =0.750 CI =25%	0/1 p-value =1.000 CI =0%	1/7 p-value =0.992 CI =0.8%	5/6 p-value =0.109 CI =89.1%
6)Clorox 1:10	4/5 p-value =0.0.187 CI =81.3%	2/2 p-value =0.250 CI =75%	2/2 p-value =0.250 CI =75%	1/2 p-value =0.750 CI =25%	7/7 p-value =0.008 CI =99.2%	No Data
7)Clorox Clean-up	5/5 p-value =0.031 CI =96.9%	No Data	Pure water not used in dilution	2/2 p-value =0.250 CI =75%	6/7 p-value =0.062 CI =93.8%	No Data
8)Vesphene IIse	1/5 p-value =0.969 CI =3.1%	2/3 p-value =0.500 CI =50%	0/2 p-value =1.000 CI =0%	0/1 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
9)70% isopropyl alcohol	3/5 p-value =0.500 CI =50%	3/3 p-value =0.125 CI =87.5%	Pure water not used in dilution	1/2 p-value =0.750 CI =25%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
10)70% ethanol	5/5 p-value =0.031 CI =96.9%	2/3 p-value =0.500 CI =50%	Pure water not used in dilution	1/2 p-value =0.750 CI =25%	7/7 p-value =0.008 CI =99.2%	6/6 p-value =0.016 CI =98.4%
11)3% hydrogen peroxide	0/5 p-value =1.000 CI =0%	0/3 p-value =1.000 CI =0%	Pure water not used in dilution	0/2 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
12)Clorox disinfecting spray	5/5 p-value =0.031 CI =96.9%	2/3 p-value =0.500 CI =50%	Pure water not used in dilution	1/2 p-value =0.750 CI =25%	7/7 p-value =0.008 CI =99.2%	6/6 p-value =0.016 CI =98.4%
13)Lysol disinfecting spray	5/5 p-value =0.500 CI =50%	3/3 p-value =.125 CI =87.5%	Pure water not used in dilution	½ p-value =0.750 CI =25%	6/7 p-value =0.062 CI =93.8%	6/6 p-value =0.016 CI =98.4%
14)TBQ	0/5 p-value =1.000 CI =0%	0/3 p-value =1.000 CI =0%	0/2 p-value =1.000 CI =0%	0/2 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
15)Povidone-iodine	0/5 p-value =1.000 CI =0%	1/3 p-value =0.875 CI =12.5%	Pure water not used in dilution	0/2 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
16)Dettol 1:20	0/5 p-value =1.000 CI =0%	0/3 p-value =1.000 CI =0%	0/2 p-value =1.000 CI =0%	0/2 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
17)Dettol 1:40	0/5 p-value =1.000 CI =0%	0/3 p-value =1.000 CI =0%	0/2 p-value =1.000 CI =0%	0/2 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
18)4% CHG	0/5 p-value =1.000 CI =0%	0/3 p-value =1.000 CI =0%	Pure water not used in dilution	0/2 p-value =1.000 CI =0%	1/7 p-value =0.992 CI =0.8%	1/6 p-value =0.984 CI =1.6%
19)Medicated Soft and Sure	0/5 p-value =1.000 CI =0%	1/3 p-value =0.125 CI =87.5%	Pure water not used in dilution	0/2 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%
20)Acute Kare	0/5 p-value =1.000 CI =0%	0/3 p-value =1.000 CI =0%	Pure water not used in dilution	0/2 p-value =1.000 CI =0%	0/7 p-value =1.000 CI =0%	0/6 p-value =1.000 CI =0%

**Probability that the Log$_{10}$ Reductions of Viral Titer for an individual germicide is at least 3.0*

***X /Y = # trials providing at least a 3.0 log reduction/ total # of trials*

Appendix Table 9.7: A Statistical Comparison of Adenovirus Titer Reductions with Variable Conditions, Table 1

A Comparison of Adenovirus Titer Reductions, Table 1					
Disinfectant	**A2:** 1min vs. 5min	**A8:** 1min vs. 5min	**A2 vs. A8** 1 min	**A2:** Hard vs. Pure water 1 min	**A2:** Hard vs. Hard & Organic Load 1 min
1)Steris Sterilant 20	1/1 for 5 min (6 indet) p =0.000	10/10 for 5 min (4 indet) p =0.001	25/25 for A2 (10 indet) p =0.000	1/6 for Pure (4 indet) p =0.984	2/4 for Hard (6 indet) p =0.688
2)Cidex-OPA	No Data	No Data	4/4 for A2 (31 indet) p =0.062	Pure water not used in dilution	4/4 for Hard (6 indet) p =0.062
3)Cidex	No Data	No Data	0/6 for A2 (29 indet) p =1.000	Pure water not used in dilution	0/2 for Hard (8 indet) p =1.000
4)Wavicide-01	No Data	No Data	35 indet	Pure water not used in dilution	10 indet
5)Clorox 1:50	6/6 for 5 min (0 indet) p =0.016	37/41 for 5 min (1 indet) p =0.000	23/34 for A2 (1 indet) p =0.029	4/9 for Pure (1 indet) p =0.746	3/5 for Hard (0 indet) p =0.500
6)Clorox 1:10	2/6 for 5 min (4 indet) p =0.891	No Data	0/7 for A2 (28 indet) p =1.000	2/2 for Pure (8 indet) p =0.25	4/6 for Hard (4 indet) p =0.343
7)Clorox Clean-up	No Data	No Data	5/5 for A2 (30 indet) p =0.031	Pure water not used in dilution	10 indet
8)Vesphene IIse	14/15 for 5 min (0 indet) p =0.0004	35/40 for 5 min (2 indet) p =0.000	24/33 for A2 (2 indet) p =0.006	5/10 for Pure (0 indet) p =0.623	3/5 for Hard (0 indet) p =0.500
9)70% isopropyl alcohol	10/13 for 5 min (2 indet) p =0.046	19/40 for 5 min (2 indet) p =0.682	28/35 for A2 (0 indet) p =0.000	Pure water not used in dilution	6/9 for Hard (1 indet) p =0.253
10)70% ethanol	8/13 for 5 min (2 indet) p =0.290	6/9 for 5 min (33 indet) p =0.253	4/14 for A2 (21 indet) p =0.971	Pure water not used in dilution	5/8 for Hard (2 indet) p =0.363
11)3% hydrogen peroxide	13/15 for 5 min (0 indet) p =0.004	21/37 for 5 min (5 indet) p =0.256	24/35 for A2 (0 indet) p =0.020	Pure water not used in dilution	2/9 for Hard (1 indet) p =0.980
12)Clorox disinfecting spray	6/11 for 5 min (4 indet) p =0.500	42 indet	0/11 for A2 (24 indet) p =1.000	Pure water not used in dilution	9/9 for Hard (1 indet) p =0.001
13)Lysol disinfecting spray	15/15 for 5 min (0 indet) p =0.000	18/19 for 5 min (23 indet) p =0.000	5/29 for A2 (6 indet) p =0.999	Pure water not used in dilution	5/7 for Hard (3 indet) p =0.226
14)TBQ	12/15 for 5 min (0 indet) p =0.017	14/33 for 5 min (9 indet) p =0.851	20/31 for A2 (4 indet) p =0.075	8/10 for Pure (0 indet) p =0.055	5/9 for Hard (1 indet) p =0.500
15)Povidone-iodine	5/15 for 5 min (0 indet) p =0.940	16/36 for 5 min (6 indet) p =0.797	22/32 for A2 (3 indet) p =0.025	Pure water not used in dilution	8/9 for Hard (1 indet) p =0.019
16)Dettol 1:20	15/15 for 5 min (0 indet) p =0.000	14/38 for 5 min (4 indet) p =0.963	12/32 for A2 (3 indet) p =0.945	8/9 for Pure (1 indet) p =0.019	4/10 for Hard (0 indet) p =0.828
17)Dettol 1:40	8/10 for 5 min (0 indet) p =0.055	17/36 for 5 min (6 indet) p =0.691	13/30 for A2 (5 indet) p =0.819	4/8 for Pure (2 indet) p =0.636	3/10 for Hard (0 indet) p =0.945
18)4% CHG	10/12 for 5 min (3 indet) p =0.019	19/30 for 5 min (5 indet) p =0.100	15/31 for A2 (4 indet) p =0.639	Pure water not used in dilution	4/8 for Hard (2 indet) p =0.636
19)Medicated Soft and Sure	15/15 for 5 min (0 indet) p =0.000	12/26 for 5 min (16 indet) p =0.721	34/35 for A2 (0 indet) p =0.000	Pure water not used in dilution	9/10 for Hard (0 indet) p =0.010
20)Acute Kare	8/13 for 5 min (2 indet) p =0.290	10/31 for 5 min (11 indet) p =0.985	17/30 for A2 (5 indet) p =0.292	Pure water not used in dilution	4/9 for Hard (1 indet) p =0.623
X/Y: indicates # of log reductions for X that were greater, than log reductions for a comparitive variable, Z X + Z = Y					

Appendix Table 9.8: A Statistical Comparison of Adenovirus Titer Reductions with Variable Conditions, Table 2

A Comparison of Adenovirus Titer Reductions, Table 2

Disinfectant	*P(A2-5min > A2-1min)	*P(A8-5min > A8-1 min)	*P(A2 > A8) 1 min	*P(Pure > Hard) A2; 1 min	*P(No Organic Load > Org Load) A2; 1 min; Hard Water
1)Steris Sterilant 20	100%	99.9%	100%	1.6%	31.2%
2)Cidex-OPA	No Data	No Data	93.8%	Pure water not used in dilution	93.8%
3)Cidex	No Data	No Data	0%	Pure water not used in dilution	0%
4)Wavicide-01	No Data	No Data	No Data	Pure water not used in dilution	No Data
5)Clorox 1:50	98.4%	100%	97.1%	25.4%	50%
6)Clorox 1:10	10.9%	No Data	0%	75%	65.7%
7)Clorox Clean-up	No Data	No Data	96.9%	Pure water not used in dilution	No Data
8)Vesphene Iise	100%	100%	99.4%	37.7%	50%
9)70% isopropyl alcohol	95.4%	31.8%	100%	Pure water not used in dilution	74.7%
10)70% ethanol	71%	74.7%	2.9%	Pure water not used in dilution	63.7%
11)3% hydrogen peroxide	99.6%	74.4%	98%	Pure water not used in dilution	2%
12)Clorox disinfecting spray	50%	No Data	0%	Pure water not used in dilution	99.9%
13)Lysol disinfecting spray	100%	100%	0.1%	Pure water not used in dilution	77.4%
14)TBQ	98.3%	14.9%	92.5%	94.5%	50%
15)Povidone-iodine	6%	20.3%	97.5%	Pure water not used in dilution	98.1%
16)Dettol 1:20	100%	3.7%	5.5%	98.1%	17.2%
17)Dettol 1:40	94.5%	30.9%	18.1%	36.4%	5.5%
18)4% CHG	98.1%	90%	36.1%	Pure water not used in dilution	36.4%
19)Medicated Soft and Sure	100%	27.9%	100%	Pure water not used in dilution	99%
20)Acute Kare	71%	1.5%	70.8%	Pure water not used in dilution	37.7%

**Probability that the Log_{10} Reductions of Viral Titer for X > the Log_{10} Reductions of Viral Titer for Z P(X>Z)*

*** All values are Confidence Intervals (CI)*

Appendix Table 9.9: A Statistical Comparison of Adenovirus Titer Reductions with Variable Conditions, Table 3

A Comparison of Adenovirus Titer Reductions, Table 3					
	***P(A2-5min > A2-1min)**	***P(A8-5min > A8-1 min)**	***P(A2 > A8)** 1 min	***P(Pure > Hard)** A2; 1 min	***P(No Organic Load > Org Load)** A2; 1 min; Hard Water
Comparison of p-values for each disinfectant per category	**12/14 for P(A2-5 min) 5 indet ****1 Disinfectant Excluded: #1*	6/13 for P(A8-5min) 6 indet *1 Disinfectant Excluded: #1*	9/16 for P(A2) 1 indet *3 Disinfectants Excluded: #1,2,3*	2/6 for P(Pure) 0 indet *1 Disinfectant Excluded: #6*	7/12 for P(No Organic Load) 5 indet *3 Disinfectants Excluded: #1,2,3*
Overall Category p-value	p=0.006	p= 0.709	p=0.402	p=0.891	p=0.387
Overall CI's	99.4%	29.1%	59.8%	10.9%	61.3%

**Probability that the Log_{10} Reductions of Viral Titer for X > the Log_{10} Reductions of Viral Titer for Z P(X>Z)*
***X/Y = # of CI's> 50% in each category/ Total # of CI's*
****Only disinfectant p-values with at least 5 determinant comparisons were used*

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