Inactivation of Surrogate Coronaviruses on Hard Surfaces by Healthcare Germicides

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ABSTRACT

Rachel Hulkower: Inactivation of Surrogate Coronaviruses on Hard Surfaces by Healthcare Germicides
(Under the direction of Mark D. Sobsey)

The SARS coronavirus (SARS-CoV) was identified as the cause of severe acute respiratory syndrome (SARS) following the worldwide 2003 outbreak. The spread of SARS in healthcare settings and the presence of SARS CoV nucleic acids on hospital surfaces in outbreak settings suggest that surfaces could potentially play a role in the spread of SARS in healthcare environments. Disinfection of hospital surfaces may interrupt virus transmission, but there are little data on the effectiveness of germicides commonly used in healthcare against coronaviruses on surfaces. The goal of this research was to test the efficacy of six standard healthcare germicides for inactivation of surrogate coronaviruses on environmental surfaces. The germicides tested were (1) Steris Vesphene® IIse Non-sterile Disinfectant Cleaner (9.09% o-phenylphenol, 7.66% p-tertiary amylphenol), (2) 70% ethanol, (3) chlorine bleach (6.0% sodium hypochlorite), (4) Cidex® OPA (ortho-phthalaldehyde), (5) Purell® Instant Hand Sanitizer (62% ethanol), (6) Clorox® Anywhere Hand Sanitizing Spray (71% ethanol). These were tested for virucidal activity against two surrogate coronaviruses, Mouse Hepatitis Virus (MHV) and Transmissible Gastroenteritis Virus (TGEV). MHV and TGEV were dried onto stainless steel surfaces, exposed to a germicide at its use dilution for one-minute contact time, and assayed for infectivity in cell culture. For TGEV, $\log_{10}$ infectivity reductions were: 70% ethanol = 3.2;

phenolic = 2.0; Cidex OPA= 2.3; 1:100 chlorine bleach = 0.35; 62% ethanol = 4.0; and 71% ethanol = 3.5. For MHV, they were: 70% ethanol = 3.9; phenolic, = 1.3; Cidex OPA = 1.7; 1:100 chlorine bleach = 0.62; 62% ethanol = 2.7; and 71% ethanol = 2.0. Of the healthcare germicides tested, only the ethanol effectively reduced infectivity of the two coronaviruses by >3 $\log_{10}$ after an exposure time of one minute. Cidex OPA was somewhat effective, with >2 $\log_{10}$ reduction, and 1:100 chlorine bleach was ineffective, with <1 $\log_{10}$ reduction. MHV and TGEV were reduced to a similar extent (within 0.5 $\log_{10}$) by the germicide tested. The results of this study suggest the need for care in choosing effective germicides against viruses like SARS CoV.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Nosocomial, or hospital-acquired, infections are responsible for thousands of deaths worldwide each year (Rutala, Peacock et al. 2006) and place financial and organizational burdens on hospitals and other healthcare facilities. Approximately 5% of all nosocomial infections are due to viral exposure (Aitken and Jeffries 2001), and in pediatric wards, viruses account for at least 30% of nosocomial infections (Sattar 2004). Surveillance and monitoring of nosocomial viral infection is limited, however, and these data are likely underestimates.

The majority of hospital-acquired viral infections are a result of exposure to respiratory or enteric pathogens (Sattar and Springthorpe 1999). Respiratory infection spreads through direct or indirect contact with contaminated droplets expelled from an infected patient. Neighboring patients and healthcare workers then become exposed to and transfer pathogens on environmental surfaces and hands (Aitken and Jeffries 2001; Varia, Wilson et al. 2003). Studies have shown viruses to be common in healthcare environments and capable of surviving for extended periods of time on environmental surfaces (Sattar 2004). In these settings, healthcare workers, medical devices, and environmental surfaces can act as both a reservoir for infection and a mode of transmission of infection to patients and staff (Cozad and Jones 2003; Rutala, Peacock et al. 2006).

In 2003, the nosocomial transmission of viral disease proved to be a major contributor to a worldwide outbreak. Early that year, a previously unknown

respiratory disease, severe acute respiratory syndrome (SARS), emerged in Guangdong Province, China (Ksiazek, Erdman et al. 2003). Studies to identify the causative agent for SARS indicated a new human coronavirus (SARS-CoV), distinct from any of the previously identified groups of coronaviruses (Drosten, Gunther et al. 2003; Peiris, Lai et al. 2003; Knobler, Mahmoud et al. 2004). Unique in its ability to spread rapidly through healthcare facilities and infect patients, staff, visitors, and volunteers, SARS-CoV was also found to be present on environmental surfaces in hospitals (Dowell, Simmerman et al. 2004), and studies demonstrated that it could survive on surfaces for 24-72 hours (Hota 2004). The outbreak of SARS highlighted the need for effective and quick evaluation of means for controlling the spread of nosocomial infection (Berger, Drosten et al. 2004).

Infection control requires effective measures for interrupting the transmission of infectious agents. Because of the importance of viruses such as SARS-CoV as causes of nosocomial infection, recent studies have focused on chemical disinfection as a means for interrupting the cycle of transmission of these viruses. Disinfection of hospital surfaces is an effective measure in reducing the risk of exposure for healthcare workers and patients (Dettenkofer and Spencer 2007); appropriate disinfection of contaminated surfaces and equipment is crucial in interrupting the spread of viruses like SARS-CoV (Ansari, Springthorpe et al. 1991; Sehulster and Chinn 2003; Hota 2004; Sattar 2007). However, to assist in the selection of appropriate germicidal agents for use against coronaviruses on hospital surfaces, data are needed on the effectiveness of commonly used hospital germicides against coronaviruses. Therefore, this study was undertaken with the following aims:

- Evaluate eight chemical germicides commonly used in healthcare settings for their efficacy in reducing infectivity of coronaviruses on environmental surfaces using two non-human coronaviruses, Mouse Hepatitis virus (MHV) and Transmissible Gastoenteritis virus (TGEV), as surrogate representatives of the *Coronaviridae* family and of human coronavirus pathogens such as SARS-CoV.
- Determine which of these germicides are effective for inactivating infectious coronaviruses on hospital surfaces.

2. OBJECTIVES

The study objectives are:

- To determine the efficacy of reduction of coronaviruses by 8 hospital surface germicides
    - Quantify reduction of infectivity of two surrogate coronaviruses, Mouse Hepatitis Virus (MHV) and Transmissible Gastroenteritis Virus (TGEV), on an environmental surface
- To provide recommendations for effective surface germicide use against coronaviruses in a healthcare setting
- To identify the advantages and disadvantages of using MHV or TGEV as a surrogate coronavirus for disinfection study

3. LITERATURE REVIEW

Nosocomial viral infections

As global travel becomes easier and world populations increase, there has been increasing concern about health and economic impacts of nosocomial viral infections (Sattar 2004). Nosocomial infections are infections that develop as a result of treatment in a healthcare facility or hospital or are caused by pathogens acquired during hospitalization (Aitken and Jeffries 2001). Also referred to as hospital- or healthcare-acquired infections, they are a significant cause of morbidity and mortality in hospitals and pose a considerable risk to patients (Rutala, White et al. 2006; Dettenkofer and Spencer 2007). It is estimated that nosocomial infections cause more than 19,000 deaths per year while contributing to another 58,000 (Rutala, Barbee et al. 2000).

Viruses are increasingly important among infectious agents affecting human health (Sattar and Springthorpe 1996; Sattar 2006). Approximately 5% of all nosocomial infections are due to viruses, but this is likely an underestimate due to a lack of standardized study and monitoring of nosocomial viral infection incidence rates (Aitken and Jeffries 2001). Viruses are the pathogenic agent responsible for most infections acquired indoors, and transmission can occur via several pathways (Boone and Gerba 2007), including direct or indirect contact, airborne droplets, or by vehicles such as food, water, medication, medical equipment, and blood (Berger, Drosten et al. 2004).

Hospitals, nursing homes, and other crowded institutional settings provide optimal conditions for an outbreak of infection because of their crowded living conditions and constant flow of human-to-human contact (Sattar and Springthorpe 1999; Aitken and Jeffries 2001). Patient-to-patient spread is an important mode of viral transmission; however, healthcare providers, staff members, and visitors are also involved in the spread of viral infection (Aitken and Jeffries 2001). Additionally, environmental surfaces serve as a mode for transmission of viral pathogens in healthcare facilities. Environmental surfaces include "medical devices, food contact surfaces, laundry, dialysis machines, ventilation systems, patient care items, and patient room surfaces" (Rutala, Barbee et al. 2000; Cozad and Jones 2003). Transmission of viral infection is dependent on the interactions between host and environment (Boone and Gerba 2007). Two main factors promote nosocomial infection in healthcare environments: a high prevalence of pathogenic organisms and a high prevalence of predisposed patients with weakened immune systems or broken skin or mucous membranes. Viral outbreaks are common because of these factors and the ease with which these modes of transmission occur in healthcare environments. Pediatric wards and wards with elderly patients are at greatest risk of nosocomial spread of viral infection; viruses account for approximately 32% of nosocomial infection among pediatric patients (Aitken and Jeffries 2001).

Viruses that spread in healthcare settings

The majority of nosocomial viral infections reported are respiratory and enteric infections; however, bloodborne viruses can also spread in healthcare environments (Sattar and Springthorpe 1999; Aitken and Jeffries 2001).

Blood-borne viruses

Blood-borne viruses are viruses that are transmitted through blood, bodily fluids, or infected tissue. The risk of blood-borne transmission is comparably lower than airborne and fecal-oral transmission, as this type of transmission generally occurs only when decontamination of medical equipment is deficient (Aitken and Jeffries 2001) or infected blood is used for transfusion.

Viruses transmitted by fecal-oral route

The fecal-oral route is the principal mode of transmission of enteric viruses. However, contact with aerosols, surfaces, and objects contaminated with enteric viruses increases the risk of transmission (Aitken and Jeffries 2001).

Viruses transmitted by respiratory route

While the frequency of nosocomial respiratory tract infections is generally underestimated, viral respiratory tract infections account for most of the nosocomial infection occurring in pediatric wards (Hall 1981). Respiratory viruses are a significant threat in healthcare environments because they spread easily via airborne droplets and person-to-person contact (Aitken and Jeffries 2001). Small droplets containing viral particles are expelled from an infected patient by coughing, sneezing, and talking and can travel significant distances. Person-to-person transmission is due to the travel of large droplets and requires direct contact with the patient (Aitken and Jeffries 2001; Varia, Wilson et al. 2003; Hota 2004). Additionally, respiratory viruses have relatively short incubation periods, allowing for the colonization of and re-transmission to new hosts (Aitken and Jeffries 2001).

Aitken and Jeffries (2001) reviewed the primary viral agents responsible for nosocomial infection:

Respiratory transmission
- Respiratory Syncytial Virus
- Influenza viruses A and B
- Parainfluenza virus, types 1,2,3
- Rhinoviruses
- Coronaviruses
- Adenovirus
- Measles, mumps, and rubella viruses

Fecal-oral transmission
- Rotavirus
- Small round-Structured Viruses (SRSVs): calicivirus; astrovirus;
- Enteroviruses
- Hepatitis A virus
- Hepatitis E virus
- Herpesviruses

Blood-borne viruses
- Hepatitis B virus
- Hepatitis C virus
- Human Immunodeficiency virus

Environmental surfaces in health care settings

While vehicles for virus transmission such as water, food, and air are well studied, there is less work on the role of animate and inanimate surfaces in viral transmission (Sattar and Springthorpe 1996). However, it has been demonstrated that environmental surface contamination contributes to the transmission of nosocomial pathogens, especially for respiratory and enteric viruses, because surfaces act as a vehicle for microorganisms (Abad, Pinto et al. 1997; Rutala, Barbee et al. 2000; Sattar 2004). Recent studies provide evidence that pathogens can persist on environmental reservoirs (Hota 2004; Dettenkofer and Spencer 2007). Viruses in various families have been shown to survive on environmental surfaces for several hours to several days (Aitken and Jeffries 2001; Sattar 2004; Boone and Gerba 2007).

Surface contamination can occur by direct or indirect contact with viral particles. Droplets containing virus are expelled from an infected patient, become airborne, and can directly land on a surface. Indirect transmission results from hands or equipment contaminated with expelled viral droplets (Varia, Wilson et al. 2003; Dowell, Simmerman et al. 2004). The Centers for Disease Control and Prevention (CDC) designate contact transmission as one of the main routes of microbial spread (Cozad and Jones 2003). Healthcare workers are an important source of contact transmission. Staff members in these environments are indicated as important animate surfaces for virus transmission because of their rapid contact with a large number of patients (Aitken and Jeffries 2001).

Inanimate surfaces can become contaminated by direct or indirect contact with pathogenic organisms. Inanimate objects that become contaminated with infectious microorganisms and serve in the transmission of these microorganisms are also known as fomites (Boone and Gerba 2007). It is important to consider the properties of fomite surfaces when assessing their role in transmission (Boone and Gerba 2007). Important characteristics include porosity and microtopography, which can affect the ability of the virus to stick to a surface as well as the ability of a germicide to interrupt virus transmission. Additionally, a surface that is not easily wettable can reduce the efficacy of a germicide for interrupting transmission (Sattar and Springthorpe 1996).

Several studies were done to identify the primary healthcare surfaces on which nosocomial viruses are found. Boone and Gerba (2007) created a list of objects that includes cloth gowns, rubber gloves, facial tissue, sponges, hands,

doorknobs, faucets, desks, phones, countertops, and computer keyboards and mouses. Further study has identified computer keyboards as important surfaces for viral transmission because of the potential number of healthcare workers coming into contact with shared computers (Rutala, Peacock et al. 2006). Other surfaces identified as priority for control of viruses by disinfection are metal surfaces (Fraise 1999), medical instrument carts, medical equipment surfaces and housekeeping surfaces (Weber and Rutala 2003).

Interruption of viral transmission with chemical disinfection

A contaminated surface can easily transfer infectious virus (Boone and Gerba 2007) , making interruption of viral transmission with chemical disinfection an important infection control measure. Disinfection is defined as a process that eliminates many or all pathogenic microorganisms, with the exception of bacterial spores, from inanimate objects (Rutala 1996; Rutala, Gergen et al. 2007). Inadequate disinfection practices account for several reported occurrences of nosocomial viral outbreaks (Aitken and Jeffries 2001).

Disinfection practices are based on the assumption that the risk of becoming infected is dependent on the number of viral particles present. Thus, effective use of chemicals for disinfection to decrease viral titer will lower the risk of infection from contact with a surface or object (Best, Springthorpe et al. 1994; Sattar 2004). Removing viral particles from an environmental surface reduces risk of infection by interrupting surface-to-human transmission (Abad, Pinto et al. 1997; Rutala, Barbee et al. 2000; Sattar 2004; Dettenkofer and Spencer 2007). Because proper and regular disinfection will most directly decrease the presence of pathogens on environmental

surfaces, it is beneficial to interfere with transmission of viruses that are present on inanimate objects, hands, and other surfaces (Weber and Rutala 2003).

Testing for efficacy of virucidal chemicals lacks reproducibility and quantitation (Best, Springthorpe et al. 1994). However, Cozad and Jones (2003) provide evidence that effective interruption of pathogenic transmission occurs when using chemicals including quaternary ammonium, iodine, alcohol, aldehyde, organic acid, peroxide, and halogenated compounds. Additionally, Sattar et al. (Sattar, Jacobsen et al. 1994) determined that chlorine, phenolic, and phenol/ethanol products were effective in reducing the titer of rotavirus dried onto stainless steel disks. These same chemicals were found to be effective in interrupting the spread of rhinovirus Type 14 as well (Sattar, Jacobsen et al. 1993).

There is very little standardization in the protocol for virucidal efficacy testing, making it difficult to compare results from different studies (Bellamy 1995). Because of this, there is little evidence to support recommendations for proper protocols for disinfection in healthcare facilities (Dettenkofer and Spencer 2007). A standardized protocol would allow for reproducible testing of germicides. The American Society for Testing and Materials (ASTM) provides technical standards for methods and materials and recommends the use of a carrier test for virucidal efficacy trials that includes drying virus onto a carrier, exposing the virus to a germicide, and controlling the contact time by dilution or neutralization (Bellamy 1995). Previous study techniques involved immersion of a disc carrier containing dried virus on its surface and a floating technique where viral film is placed on top of liquid germicides.

Hospital standards

E.H. Spaulding created a classification system for disinfection of medical equipment based on the degree of risk of infection when an instrument is used for patient care. Classification is divided into three categories: critical, semicritical, and noncritical items:

- Critical items involve a high level of risk for infection if contaminated with a pathogen. These items are used to enter sterile tissues and the vascular system.
- Semicritical items are objects that have direct contact with broken skin or mucous membranes.
- Noncritical items only come into contact with intact skin.

Germicides can also be classified based on the extent to which a chemical kills microorganisms present on a surface or fomite.

- High-level classification indicates that a chemical is expected to destroy all microorganisms, excluding bacterial spores.
- Intermediate-level chemicals will inactivate *Mycobacterium tuberculosis*, vegetative bacteria, and most viruses and fungi.
- Low-level disinfection can kill most bacteria and some viruses and fungi (Rutala 1996; Rutala and Weber 1999).

Survival and disinfection of coronaviruses

Severe Acute Respiratory Syndrome

The discovery of severe acute respiratory syndrome (SARS) and the realization of the global health impacts of nosocomial outbreaks caused by SARS coronavirus (SARS-CoV) created considerable interest in virus disinfection research (Sattar 2004). The newly emerged SARS virus was responsible for approximately 8000 cases and 700 deaths worldwide before the chain of natural transmission was broken (Berger, Drosten et al. 2004; Rabenau, Cinatl et al. 2005). When SARS was recognized as a disease caused by an unidentified infectious agent, the World Health

Organization (WHO) led efforts to identify the causative agent, and on 16 April, 2003 the agent was identified as a novel coronavirus (Drosten, Gunther et al. 2003; Dwosh, Hong et al. 2003; Ksiazek, Erdman et al. 2003; Peiris, Lai et al. 2003; Varia, Wilson et al. 2003; Berger, Drosten et al. 2004).

SARS-CoV is unique in its ability to cause hospital outbreaks and rapidly spread over a large geographical area. Unlike influenza, SARS-CoV affected immunocompetent, mobile individuals as well as elderly and immunocompromised populations, so the virus was able to spread worldwide very quickly (Berger, Drosten et al. 2004). Although the first cases were reported in November of 2002, the global outbreak of SARS-CoV originated from a single individual in Guangdong, China (Berger, Drosten et al. 2004). One of the people infected by this patient visited a hospital resulting in an outbreak affecting 88 healthcare workers and 18 medical students (Chan-Yeung 2003). In total, 30 counties, including the United States, Canada, China, and Singapore, reported SARS cases (Berger, Drosten et al. 2004).

Several factors contributed to the ability of SARS to spread quickly. SARS-CoV has a short to intermediate incubation period ranging from 2 to 16 days, with a median of 6 days. Additionally, the time period between infection and onset of symptoms varies considerably, making early diagnosis and treatment difficult (Berger, Drosten et al. 2004). There is also evidence that the SARS-CoV is able to survive for several hours on environmental surfaces (Dowell, Simmerman et al. 2004).

Transmission of SARS-CoV is primarily by droplets over short distances, however, direct and indirect contact with contaminated respiratory secretions also

encourages transmission (Dwosh, Hong et al. 2003). In addition to respiratory droplets and contaminated surfaces, the virus spreads through fecal-oral transmission (Hota 2004). The droplets can contaminate environmental surfaces in hospitals, so fomites are considered to be a possible mode of transmission of SARS. During the outbreak in 2003, healthcare workers continued to contract SARS even after barrier precautions were employed. It is believed that healthcare workers continued to become exposed to the virus because of its presence on hospital surfaces (Dowell, Simmerman et al. 2004).

Previous studies on virus disinfection have resulted in a number of recommendations for future study. The theory that disinfection of environmental surfaces will reduce surface contamination (Hota 2004), thus reducing transmission, has been tested using several study protocols. Many disinfection studies are done using suspension tests (Rutala, Barbee et al. 2000); however, Sattar et al. (2003) stress the need for standardized testing methods and recommend carrier testing, using viruses dried on a surface. The carrier-based test method more closely mimics "real life" situations where germicides are used (Bellamy 1995). In this method the inoculum is exposed to germicide chemicals after being dried onto a model surface. Since viruses are naturally found adsorbed to surfaces, data gained from carrier methods may best describe expected results in daily use (Sattar, Springthorpe et al. 2003). Experimental conditions, including use-dilutions and contact times, are chosen based on the way germicides are actually used by staff in healthcare settings.

When the virus in question is highly infectious and requires laboratory containment conditions that are difficult to obtain, surrogate viruses should be used

(Steinmann 2004). There are several factors to consider when choosing a surrogate virus. Candidate surrogate viruses should represent different families and groups and be more resistant to disinfection than the pathogenic virus of interest (Bellamy 1995; Abad, Pinto et al. 1997).

Surface disinfection of SARS-CoV and other coronaviruses as a means for interrupting transmission is an important focus for research. Rabenau et al. (2005) studied the survival and inactivation of SARS-CoV when compared to another human coronavirus, HCoV-229E, using both suspension and carrier testing. When dried onto experimental surfaces, HCOV-229E survived for 24 hours and SARS-CoV survived for 6 days. It was concluded that SARS-CoV is more stable in the environment than other human coronaviruses. This study also tested common hand and chemical germicides for their efficacy against SARS-CoV. Desderman (96% ethanol + 2-biphenylol) reduced infectivity by $>5.01\log_{10}$; Sterillium (80% ethanol) resulted in $>2.78\log_{10}$ reduction. Seventy percent and 100% isopropanol both achieved $>3.31\log_{10}$ reductions after 30 seconds contact time. The chemical germicides tested were formaldehyde, glutardialdehyde, Incidin plus (2% glycoprotamin), and wine vinegar, which resulted in $>3.01\log_{10}$, $>4.01\log_{10}$, $>1.68\log_{10}$, and $\geq 3.01\log_{10}$ reductions, respectively (Rabenau, Cinatl et al. 2005).

Comparison of Efficacy trials

Table 3.1.1 Virucidal Activity for Previously Studied Germicides.

Active Ingredient	Concentration (%)	Method	Reduction of virus titer >99.9% HCV	Ad5
Hypochlorite	0.01	Carrier	No	No
	0.10	Carrier	Yes	No
	0.50	Carrier	Yes	Yes
	1.00	Carrier	n.d.	Yes
Organochlorine	0.01	Carrier	No	No
	0.10	Carrier	Yes	No
	0.30	Carrier	Yes	Yes
	0.50	Carrier	n.d.	Yes
Mixed Halide	0.01	Carrier	No	No
	0.05	Carrier	Yes	No
	0.1	Carrier	Yes	No
Iodophore	10.00	Carrier	Yes	No
Ethanol (alcohol)	70.00	Carrier	Yes	Yes
Glutaraldehyde	2.00	Carrier	Yes	Yes
Quaternary ammonium	0.04	Carrier	No	No
Quaternary ammonium with HCl	0.04, 7	Carrier	Yes	Yes
Quatenary ammonium with ethanol	0.04, 70.0	Carrier	Yes	Yes
Quaternary ammonium with metasilicate	0.04, 0.5	Carrier	Yes	Yes
Chlorhexidine	0.008	Carrier	No	No
Chlorhexidine with ethanol	0.05, 70	Carrier	Yes	Yes
Phenolic	0.06	Carrier	No	No
Phenolic with SDS	0.06, 0.60	Carrier	Yes	No
Phenolic with ethanol	0.06, 70.0	Carrier	Yes	Yes
Phenolic with SDS	0.50, 0.60	Carrier	Yes	Yes

Source: Sattar et al. 1989
HCV: Human coronavirus 229E
Ad5: Adenovirus type 5
n.d.: not done

Active Ingredient	Use-dilution	Method	Contact Time	Log10 reduction
ortho-phthalaldehyde	undilute	Carrier	1 min	4.37
Quaternary ammonium	1:128	Carrier	1 min	0.38
Phenolic	1:128	Carrier	1 min	0.41
Isopropyl alcohol	70%	Carrier	1 min	0.95
Clorox bleach (6% sodium hypochlorite)	1:50	Carrier	1 min	1.99
Clorox bleach (6% sodium hypochlorite)	1:10	Carrier	1 min	4.87
Ethyl alcohol	70%	Carrier	1 min	4.62

Source: Rutala 2006
Virus used: Adenovirus 8

Disinfectant (Active Ingredient)	Use-dilution	Method	Contact time (min)	Log10 Reduction
Vesphene IIse	1:128	Suspension	0.5	0.033
(Phenolic)		Suspension	5	0.22
TBQ	1:128	Suspension	0.5	0.1
(Quaternary ammonium)		Suspension	5	0.09
Clorox	1:10	Suspension	0.5	>3.3
(Sodium hypochlorite)		Suspension	5	>3.3
Ethanol	70%	Suspension	0.5	0.03
		Suspension	5	0.65
Lysol Disinfectant	undilute	Suspension	0.5	>3.3
(Ethanol, quaternary ammonium)		Suspension	5	3.1
Lysol Antibacterial	undilute	Suspension	0.5	0.1
(Quaternary ammonium)		Suspension	5	0.27
Mr. Clean	undilute	Suspension	0.5	0.19
(Ionic, nonionic surfactants)		Suspension	5	0.15
Vinegar	undilute	Suspension	0.5	0.25
		Suspension	5	0.32
Baking Soda	undilute	Suspension	0.5	0.14
		Suspension	5	0.42

Source: Rutala et al. 2000
Virus used: poliovirus

Table 3.2.1 Virucidal Activity for Previously Studied Germicides Against SARS-CoV.

Active Ingredient	Method	Contact Time	Minimal Log10 Reduction
100% 2-Propanol	Suspension	30s	>3.31
70% 2-Propanol	Suspension	30s	>3.31
78% Ethanol	Suspension	30s	>5.01
45% 2-propanol, 30% 1-propanol	Suspension	30s	>2.78
Wine vinegar	Suspension	60s	>3.0
0.7% formaldehyde	Suspension	120s	>3.01
1.0% formaledhyde	Suspension	120s	>3.01
0.5% glutardialdehyde	Suspension	120s	>4.01
26% glucoprotamin	Suspension	120s	>1.68
Source: Rabenau 2005a			
Active Ingredient	**Method**	**Contact Time**	**Reduction Factor**
45% iso-propanol, 30% n-propanol and 0.2% mecetronium etilsulphate	Suspension	30s	>4.25
80% ethanol	Suspension	30s	>4.25
85% ethanol	Suspension	30s	>5.5
95% ethanol	Suspension	30s	>5.5
0.5% benzalkonium chloride and laurylamine	Suspension	30 min	>6.13
0.5% benzalkonium chloride, glutaraldehyde and didecyldimonium chloride	Suspension	30 min	>3.75
0.5% magnesium monoperphthalate	Suspension	30 min	>4.5
glutaraldehyde and (ethylenedioxy)dimethanol	Suspension	15 min	>3.25
Source: Rabenau 2005b			
Active Ingredient	**Method**	**Contact Time**	**Inactivation Rate (%)**
Sodium hypochlorite (10mg/L)	Suspension	1 min	43.77
		5 min	68.38
		10 min	100
		20 min	100
		30 min	100
Chlorine dioxide	Suspension	1 min	43.77
		5 min	68.38
		10 min	68.38
		20 min	68.38
		30 min	55.53
Source: Wang 2005			

There have been many previous studies on the efficacy of germicides against viruses. Tables 3.1-2 demonstrate the amount of variability in the way germicides act against viruses. The two primary methods for testing germicidal efficacy are suspension testing and carrier-based testing. As discussed earlier, the carrier-based testing is generally considered the higher quality test because it closely reproduces conditions in the field. Suspension tests are done by adding virus to solutions containing a germicide chemical that are then neutralized and assayed. Carrier-based tests involve drying virus onto test surfaces, exposing the surface to germicide and neutralizer and assaying the eluent. Surfaces used in the carrier method also vary by experiment and include glass (Best, Springthorpe et al. 1994), stainless steel (Sattar, Springthorpe et al. 2003), and Petri dishes. As evident in Table 3.1 and 3.2, the $\log_{10}$ reduction in viral infectivity is generally greater for studies using the suspension method. Because of these variations in methods for evaluating antimicrobial activity, results are also greatly varied.

4. EXPERIMENTAL APPROACH

The purpose of this study was to test the efficacy of 8 hospital surface germicides in reducing the infectivity of coronaviruses dried onto hard nonporous surfaces using two non-human coronaviruses, MHV and TGEV, chosen as candidate surrogates for SARS-CoV.

The selection of a surrogate coronavirus most closely related to SARS-CoV is difficult because genomic sequence information shows it to be genetically distinct from the currently recognized groups in the *Coronaviridae* family (Knobler, Mahmoud et al. 2004), making its placement within the family uncertain. Therefore, two coronaviruses, Mouse Hepatitis Virus (MHV) and Transmissible Gastroenteritis Virus (TGEV), were selected as candidate surrogates for SARS-CoV because they represent the two distinct antigenic groups of mammalian viruses within the *Coronaviridae* family, Group 2 and Group 1, respectively.

Six germicides commonly used for cleaning hospital surfaces were chosen as representatives of the types of germicides used in healthcare settings. The active ingredients tested included: ortho-phthalaldehyde, sodium hypochlorite, ethanol, and phenol.

The carrier-based method used to determine efficacy of reduction in viral titer was adapted from the method defined by Sattar et al. (Sattar, Springthorpe et al. 2003). Virus was dried onto stainless-steel carriers designed to simulate a typical environmental surface. Dried virus was then exposed to each chemical germicide for

one-minute contact time. Under field conditions, viruses are mostly found dried onto surfaces and embedded in bodily excretions. Drying the virus before disinfecting replicates the worst-case scenario for viral exposure (Yilmaz and Kaleta 2003) and presents a stronger challenge than disinfecting the virus in suspension (Sattar, Springthorpe et al. 2003). Virus titers before and after germicide exposure were then determined using either plaque assay to enumerate viruses or quantal assay in multi-well plates to estimate virus concentration by most probable number (MPN) techniques. This carrier-based protocol was chosen because it allowed for greater control of contact time and chemical neutralization of virucidal activity. Additionally, when compared to the suspension test method, the carrier method more closely mimics the conditions for surface contamination and surface contact-related virus transmission in the healthcare setting (Bellamy 1995). The volume and titer of virus used in the carrier-based experiments was chosen so that the inoculum closely represented the size of the contaminated droplets found in healthcare settings. The initial viral titer in the inoculum was 10^5 PFU/20μL, making it possible to observe a 3-4 $\log_{10}$ reduction in virus infectivity by the germicide.

The germicide-neutralizer combinations used in these experiments may potentially have cytotoxic effects on the mammalian cell lines used to assay viruses, leading to difficulty in interpreting infectivity assay results. Preliminary experiments based on microscopic observation of cells exposed to germicides and neutralizers were performed to ensure that the germicides and their neutralizers did not have visible cytotoxic effects on cells used for infectivity assays. Cytotoxic effects included observable cell death, rounded cells, monolayer destruction and physical

interference with reading of infectivity assays based on plaque count and viral cytopathic effects. Virus infectivity interference experiments were also performed. These experiments determine whether exposure of cells to germicides and neutralizers caused cell damage that was not visible upon microscopic observation of cells but reduced the sensitivity of infectivity assays, possibly by interfering with viral attachment and infection.

Reduction in viral titer expressed as $\log_{10}$ Nt/N0, where N0 is the virus titer at time 0 and Nt is the virus titer at time t, was used to determine the efficacy of each germicide. These data were then used to compare reduction in viral infectivity for each germicide chemical. Previous research in virus disinfection suggests that a 3 $\log_{10}$ reduction in infectivity demonstrates germicide efficacy for short contact-times (Bellamy 1995; Rutala, Barbee et al. 2000; Steinmann 2001). In addition to determining whether each germicide met the 3-log10 performance target, comparisons of efficacy in virus reduction were made between germicides. Additionally, for individual germicide chemicals, the extent of reduction in infectivity for the two test viruses was compared.

5. MATERIALS AND METHODS

5.1 Preparation of viral stocks

Mouse Hepatitis Virus (MHV) and Transmissible Gastroenteritis Virus (TGEV) were kindly provided by R. Baric, University of North Carolina, Chapel Hill. TGEV was grown in swine testicular (ST) cell cultures. MHV was grown in delayed brain tumor (DBT) cell cultures. Viral stocks were propagated by infecting confluent layers of host cell cultures in flasks, harvesting cell lysates, clarifying by centrifugation (3000×g, 30 min, 4°C), and storing resulting supernatants as virus stock at -80°C. Viral titers were determined by the plaque assay method on confluent host cell layers in 60 mm petri dishes with overlay medium consisting of 1% agarose, Eagle's minimum essential medium (MEM), 10% bovine serum replacement (Fetal Clone II, Hyclone, Logan, UT), 7.5% bicarbonate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), non-essential amino acids, 100mM sodium pyruvate, 10% lactalbumin hydrolysate and gentamicin/kanamycin (1mg/mL). Cell layers were stained with a second overlay containing 1% neutral red at 48 hours post-infection and plaques were visualized at 72 hours post-infection.

5.2 Preparation of Hard Water

The protocol for preparation of hard water was adapted from the USEPA OPP Microbiology Laboratory protocol. Hard water was prepared from two stock solutions: Solution A (14.01g of $NaHCO_3$, 250mL of sterile deionized water) and

solution B (16.94g $MgCL_2$-$6H_2O$, 18.50g $CaCl_2$, 250 mL sterile deionized water). Solution A was filter sterilized using 0.22μm pore size filters; Solution B was autoclaved at 121°C for 30 minutes.

Hard water was prepared as follows: 12 mL of solution A was placed in a 1L volumetric flask and 600mL sterile deionized water was added. Twelve mL of solution B was added, and the flask filled to the 1L mark with sterile deionized water. This mixture was then placed in a sterile 4L container and diluted with 2 additional liters of sterile water. Final solution was pH adjusted by dropwise addition of sodium hydroxide or citric acid to pH 7.6 -8.0. A hardness testing kit (Hach® Model 5-EP mg/L #1454-01, Loveland, Co.) was used to ensure that the hardness of the prepared water was 380-400mg/L $CaCO_3$.

5.3 Germicides

Eight hospital grade germicides, obtained from Dr. William Rutala, were tested for their effectiveness in reducing the infectivity of MHV and TGEV dried onto surfaces. The germicide types, active ingredients, and use-dilutions are summarized in Table 5.4.1. Germicides requiring dilution were prepared on the day of the experiment using hard water as the diluent. All germicides were replaced after the expiration date as defined by the manufacturer.

Table 5.3.1 Germicides Tested for Reduction in Infectivity

Germicide	Germicide Type	Active Ingredient	Use-dilution
Steris Vesphene® IIse Non-sterile Disinfectant Cleaner	Phenol	9.09% o-phenylphenol, 7.66% p-tertiary amylphenol	1:128
Chlorine bleach	Halogen	6% sodium hypochlorite	1:100
Cidex® OPA	Aldehyde	0.55% Ortho-phthalaldehyde	Undiluted
70% ethanol	Alcohol	70% ethanol	Undiluted
Purell ® Hand Sanitizer	Alcohol	62% ethanol	Undiluted
Clorox Anywhere Hand Sanitizing Spray	Alcohol	71% ethanol	Undiluted
Steris T.B.Q.® Hard Surface Disinfectant Cleaner	Quaternary ammonium	8% dimethyl benzyl ammonium chloride	1:128
Lysol Disinfectant Spray	Quaternary ammonium	0.106% Alkyl, dimethyl benzyl ammonium saccarinate, 79.646% Ethanol	Undiluted

5.4 Neutralizing Solutions

Neutralizing solutions were used to ensure that the active germicide chemical maintained contact with the virus for only the specified contact-time, after which it was chemically changed to make it inactive. The addition of a neutralizing solution, as outlined in section 4.9, halts virucidal activity of the germicide. Neutralizing solutions were matched to germicides based on previous research (Rutala, Peacock et al. 2006).

The neutralizing solutions used in this study were 3% glycine, 0.1% sodium thiosulfate, 0.5% sodium bisulfite. In order to prevent cytotoxicity due to exposure to neutralizing chemicals, each neutralizing solution, with the exception of 3% glycine, was prepared at higher concentration and diluted with cell culture medium. Three percent glycine was diluted by adding 2.5mL 5% cell culture medium to 47.5mL 3% glycine. A 10% sodium thiosulfate solution was prepared and diluted to 0.1% final concentration by combining 99mL cell culture medium with 1mL of a 10% (W/V) sodium thiosulfate solution. A 5% sodium bisulfite in deionized water solution was diluted with 9mL cell culture medium per 1mL of neutralizer for a final concentration of 0.5%.

Table 5.4.1 Neutralizing Solutions

Germicide	**Neutralizing Solution Before Mixing with Germicide**
Vesphene IIse	3% glycine
Chlorine bleach	0.1% thiosulfate
70% Ethanol	3% glycine
Cidex-OPA	0.5% sodium bisulfite
Clorox Anywhere Spray	3% glycine
Purell Sanitizing Hand Gel	3% glycine
TBQ	**
Lysol	**
** Indicates no neutralizing solution identified	

5.5 Cytotoxicity Testing of Germicide on DBT and ST Cell Lines

The purpose of this set of experiments was to test for cytotoxic effects of the germicides and neutralizing solutions on delayed brain tumor (DBT) and swine testicle (ST) cell monolayers. Cytotoxic effects on cell monolayers due to the germicide or neutralizer could have an effect on the ability of viral particles to attach

to cells and initiate infection, and may influence the quantification of viral concentrations after disinfection.

Cytotoxicity testing procedures were adapted from Sattar et al. (Sattar, Springthorpe et al. 2003). Confluent cell monolayers were inoculated with 200µL of six different solutions: germicide alone, a 1:10 dilution of germicide, neutralizing solution alone, 940µL neutralizing solution + 50µL germicide, and a 1:10 dilution of the germicide-neutralizer mixture. Cell monolayers were incubated at 37°C for 1 hour with shaking every 15 minutes. After one hour, the plates were removed from the incubator and observed under a microscope. Visible changes in cell monolayer were recorded. Inocula were aspirated from cell monolayers, cell culture medium were added to each well, and the plates were retuned to 37°C. Cell monolayers were examined for visible changes every 24 hours for three days.

5.6 Virus Infectivity Interference Testing of Germicides on DBT and ST Cell Lines

Virus infectivity interference testing protocols were adapted from Sattar et al. (2003). Confluent cell monolayers were inoculated with each germicide-neutralizer combination to be used in future experiments, at the dilution and contact times used for disinfection testing. After exposure to the germicide-neutralizer combination, cells were inoculated with dilution of stock virus to determine viral titer. Control titers were done on cell monolayers not exposed to germicide-neutralizer combination.

To determine if germicide-neutralizer combinations caused interference with viral infection on cell monolayers, viral titers on exposed and unexposed cell

monolayers were compared. Statistically significant differences in viral titer on exposed and unexposed cell monolayers were an indication of interference with viral infection by germicide-neutralizer combinations.

5.7 Virus Assay Protocol

For a viral plaque assay, samples were diluted in cell culture medium and inoculated onto confluent cell monolayers in 60mm petri dishes. Plates were incubated for 1 hour, shaken gently every 15 minutes (37°C, 5% CO_2). After 1 hour, inoculum was aspirated and replaced with 5mL 2% Agarose with 2X MEM and Fetal Clone II supplements (as described previously). Plates were incubated for 48 hours, a second layer of 1% Agarose containing 1% neutral red (Sigma, 3.3g neutral red/L Dubelco's Phosphate Buffered Saline) (1mL neutral red in 100mL agarose), and the plates were incubated for an additional 24 hours. After 24 hours, cell monolayers were observed and plaques were counted visually. Results were expressed as plaque forming units (PFU).

For quantal assays in cell cultures with liquid media to compute most probable number (MPN) estimates of virus concentration, samples were diluted in cell culture medium and inoculated onto confluent cell monolayers in 24-well trays. Trays were incubated for 1 hour (37°C, 5% CO_2). After 1 hour, 1mL of cell culture medium was added to each well to further dilute the inoculum. Trays were incubated for 48 hours and checked for cytopathic effects. Each well was scored as positive or negative for CPE in order to compute virus titer as MPN per ml.

5.8 Virus Elution Efficiency Testing of Neutralizing Solutions on MHV and ST Cell Lines

For each neutralizer, 20 µL of virus was dried onto the surface of three stainless steel carriers in a biological safety cabinet for a standard 2 hours. The steel carriers were placed in wells in a 24-well plate, and 950µL neutralizing solution and 150µL 15% beef extract were added to each well. The 24-well plate was agitated gently for 20 minutes on a shaker platform. Samples were diluted and assayed for viruses by plaque assay.

5.9 Disc-based Quantitative Carrier Test Method for Virus Disinfection

Each germicide experiment used three control carriers (no germicide applied) and three test carriers (germicide applied). The experiments were repeated twice for each germicide and assayed for test viruses on both cell culture lines. The carrier test method was adapted from a procedure described by Sattar et al (2003). Carriers were 1 cm^2 stainless steel with a No. 4 polish.

Each carrier was placed inside a well in a 24-well plate, and 20µL of stock virus were applied. The virus was allowed to dry for a standard two hours. After two hours, 50µL germicide was placed on three carriers for one minute of contact time. After one minute, 950µL of neutralizing solution was added to halt virucidal activity. On the control carriers, maintenance medium was substituted for both the germicide and neutralizing solutions. A 150µL eluting solution of 15% beef extract (pH 7.5) was then added to all carrier wells. Carriers were agitated gently on a shaker for 20

minutes. The eluent from each well was then recovered, diluted, and assayed for viruses as previously described.

To determine the reduction in virus infectivity, the concentration of virus per 20µL sample volume was calculated and compared to the control concentration. Both percent reduction and $\log_{10}$ reduction in viral titer were calculated from the difference in virus concentrations between elution solutions of control carriers and the germicide treated carriers.

5.10 Statistical Analysis

Statistical analysis was performed to compare the virucidal activity of six hospital surface germicides using the statistical program SAS 9.1 (SAS Institute Inc. 2008). A One-way ANOVA was done for data on each virus to compare the $\log_{10}$ reduction by each of the germicides. Additionally, a Two-way ANOVA was performed to compare the efficacy of all surface germicides between the two virus types.

6. RESULTS

6.1 Test for Cytotoxicity

Cytopathic effects (CPE) were identified by observable damage to the cell monolayer. Wells displaying no CPE had cell layers that were confluent across the bottom of the well. Observable damage to the monolayer included rounding cells, aggregates of cells detached from the monolayer and floating in the maintenance medium, irregularly shaped gaps in the monolayer, and a change in the color of the maintenance medium. Cell cultures were not expected to last longer than one week, so damage was attributed to cell culture age when observed in the control wells and 48 hours after the cells were inoculated.

Cytotoxicity testing results are recorded in Table 6.1. Cytotoxic effects were observed when each cell line was exposed to Lysol disinfectant spray, Clorox Anywhere spray, Purell hand gel, TBQ, chlorine bleach, and 70% ethanol. Cidex-OPA and Vesphene IIse produced little or no cytotoxic effect after 24 hours of exposure. Two neutralizing solutions, 3% glycine and 0.1% sodium thiosulfate, produced no cytotoxicity on the ST or DBT cell lines. The 3% glycine neutralizer was not effective in neutralizing the cytotoxicity of Lysol disinfectant spray, and the combination of the germicide and neutralizer produced cytotoxic effects after 24 hours. Cidex-OPA was tested with 3% and 5% glycine neutralizer solutions, and both of them were unsuccessful in neutralizing the germicide. Each Cidex-neutralizer combination resulted in cytotoxicity after 24 hours of exposure. Neutralization of

Clorox Anywhere spray, Purell hand gel, Vesphene IIse, and 70% ethanol was successful using 3% glycine, and no cytotoxic effects were observed.

Table 6.1.1 Test for Cytotoxicity

	Cytopathic Effects Observed							
	Bleach	Vesphene IIse	Cidex-OPA	70% Ethanol	Purell Hand Sanitizer	Clorox Anywhere Spray	Lysol	T.B.Q.
Germicide (Use-dilution)	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Germicide (1:10)	No	No	No	Yes	Yes	Yes	Yes	Yes
Neutralizer	No	No	No	No	No	No	No	No
Germicide +Neutralizer	No	No	Yes	No	No	No	Yes	Yes
Germicide (Use-dilution) + Neutralizer (1:10)	No	No	No	No	No	No	Yes	Yes

6.2. Testing for Virus Elution Efficiency

Virus elution efficiency was tested to determine if there was a loss of viral titer after the virus is dried on a stainless steel carrier, exposed to neutralizer and then eluted using 15% beef extract eluent. Three neutralizing solutions were tested: 3% glycine, 0.1% sodium thiosulfate, and 0.5% sodium bisulfite. Percent recovery and $\log_{10}$ reduction in infectious titer were calculated by comparing eluted virus titer to the titer of stock virus placed on the carrier.

For dried MHV exposed to 3% glycine solution, recovery efficiency was 44.6% (95% CI = 37.90, 51.31), or a 0.35 $\log_{10}$ (95% CI = 0.29, 0.42) loss of viral titer. For MHV exposed to 0.1% sodium thiosulfate, recovery efficiency was 79% (73, 85), or a 0.10

$\log_{10}$ (0.07, 0.14) loss of viral titer. Exposure to 0.5% sodium bisulfite resulted in a recovery efficiency of 18.6% (13.4, 23.7), or a 0.74 $\log_{10}$ (0.63, 0.85) loss of viral titer.

For dried TGEV exposed to 3% glycine solution, recovery efficiency was 36% (19.9, 53), or a 0.44 $\log_{10}$ (0.21, 0.67) loss of viral titer. For TGEV exposed to 0.1% sodium thiosulfate, recovery efficiency was 42.5% (39, 46), or a 0.37 $\log_{10}$ (0.34, 0.41) loss of viral titer. Finally, for TGEV exposed to 0.5% sodium bisulfite, recovery efficiency was 48% (36, 59), or a 0.32 $\log_{10}$ (0.20, 0.43) loss of viral titer.

Table 6.2.1 Testing for Elution Efficiency of Virus Dried on Carrier and Neutralized

Virus	MHV		TGEV	
Neutralizer	% Recovery	Log10 Reduction	% Recovery	Log 10 Reduction
3% glycine	44 (38, 51)	0.35 (0.29, 0.42)	36 (20, 53)	0.44 (0.21, 0.67)
0.1% sodium thiosulfate	79 (73, 85)	0.10 (0.07, 0.14)	43 (39, 46)	0.37 (0.34, 0.41)
0.5% sodium bisulfite	19 (13, 24)	0.74 (0.63, 0.85)	48 (36, 60)	0.32 (0.20, 0.43)

6.3 Testing for Interference with Virus Infectivity Assay

Stock virus infectivity assays were performed on cell monolayers that had been either exposed to germicide-neutralizer combinations (exposed) or were unexposed (negative control). Titers were obtained in plaque and MPN assays. To determine whether cell damage caused by germicide exposure interfered with the process of viral replication, virus titers of experimental (exposed) and negative control assays were compared. Results are shown in Table 6.3.1.

The germicide/neutralizer combinations tested did not cause a significant decrease in viral titer on exposed vs. control cell cultures. Chlorine bleach with 0.1% sodium thiosulfate, and 70% ethanol with 3% glycine did not produce a significant difference in viral titer on exposed vs. control cells for both ST and DBT cell lines.

Vesphene with 3% glycine produced a significantly higher viral titer on DBT cells, but there was no significant difference on ST cells. Ethanol-based Purell Hand gel and Clorox anywhere spray, also gave a significantly higher titer on DBT cells. However, on ST cells, both Purell hand gel and Clorox Anywhere Spray did not produce a significant difference in viral titer on exposed vs. control cells. Exposure to Cidex-OPA with 3% glycine resulted in significant damage to the cell monolayer, but Cidex-OPA with 0.5% sodium bisulfite showed no significant difference in virus titer between exposed and control cell cultures.

TBQ and Lysol were not successfully neutralized by any neutralizers tested. When combined with 3% glycine, 6% glycine, 0.5% sodium bisulfite, 2.5% sodium bisulfite, 1X Letheen broth (beef extract, Proteose Peptone No. 3, Polysorbate 80, Lecithin, Sodium chloride), 2X Letheen broth, and maintenance medium, these germicides caused significant cytotoxicity, making titer assays unreadable.

Table 6.3.1. Testing for Interference of Germicide/Neutralizing Solution with Virus Infectivity Assays (n = 6)

Germicide	Sample (Treatment)	MHV		TGEV	
		Viral titer (log_{10})	95% Confidence Interval	Viral titer (log_{10})	95% Confidence Interval
Cidex-OPA	Control	5.63	(5.49, 5.77)	5.14	(4.95, 5.34)
	Experimental	5.84	(5.71, 5.97)	4.89	(4.68, 5.09)
Vesphene	Control	6.79	(6.46, 7.11)	4.90	(4.76, 5.03)
	Experimental	7.26	(7.21, 7.32)	4.77	(4.71, 4.82)
70% Ethanol	Control	6.79	(6.46, 7.11)	4.90	(4.76, 5.03)
	Experimental	6.90	(6.80, 6.99)	4.73	(4.52, 4.95)
Chlorine bleach	Control	6.79	(6.46, 7.11)	5.14	(4.95, 5.34)
	Experimental	6.63	(6.60, 6.67)	5.04	(4.96, 5.12)
Clorox Anywhere spray	Control	6.28	(6.13, 6.43)	3.61	(3.11, 3.83)
	Experimental	6.65	(6.57, 6.72)	3.75	(3.60, 3.86)
Purell Hand Gel	Control	6.28	(6.13, 6.43)		
	Experimental	6.64	(6.62, 6.66)	4.83	(4.66, 4.96)
TBQ	Control	**	**	**	**
	Experimental	**	**	**	**
Lysol disinfectant spray	Control	**	**	**	**
	Experimental	**	**	**	**
		**	**	**	**

** No visible plaques, and host cell culture damage prevented calculation of viral titer

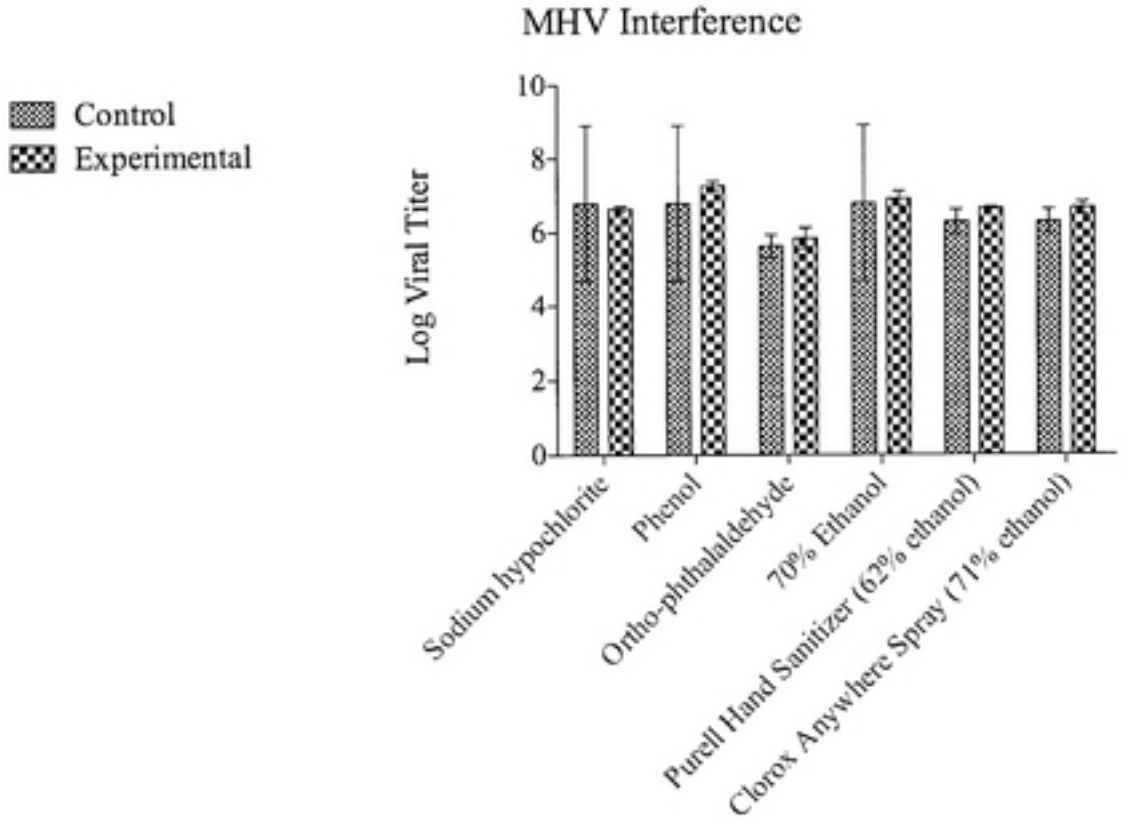
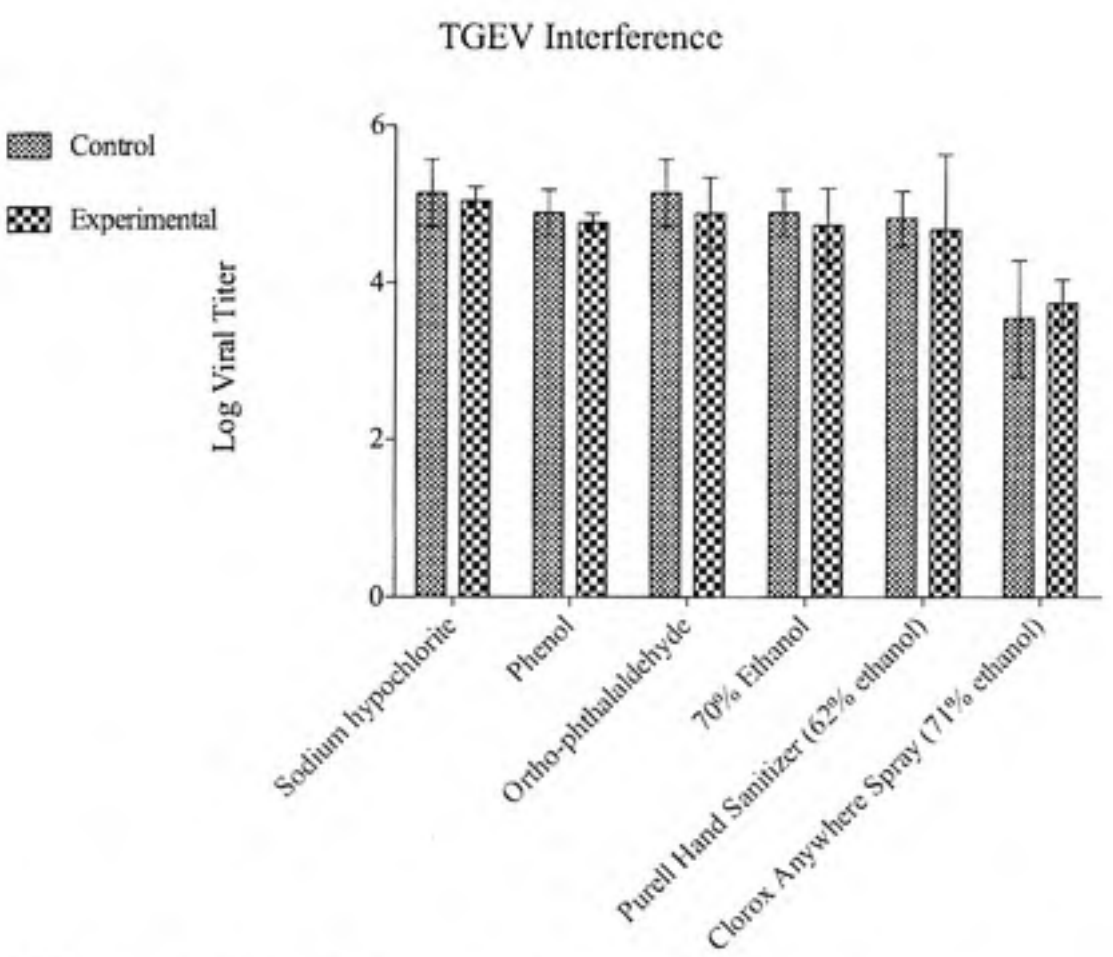


Figure 6.3.1 Mean $\log_{10}$ MHV infectivity titer of Control and Experimental samples



6.3.2 Mean $\log_{10}$ TGEV infectivity titers of Control and Experimental samples.

6.4 Testing for Germicide Efficacy Against MHV and TGEV on Carrier Surfaces

The reductions of MHV and TGEV infectivity on surfaces by hospital germicides are shown in Table 6.4.1.

Log_{10} reductions were calculated for each germicide based on six independent exposure trials. Only 70% ethanol and Purell Hand Gel (62% ethanol) produced >2.5 $\log_{10}$ reductions in infectious MHV titer. For MHV, 1:100 hypochlorite, Vesphene IIse, Cidex-OPA, and Clorox Anywhere spray each produced <2.5 $\log_{10}$ reductions, with log10 reductions of 0.62, 1.33, 1.71, and 1.98, respectively. Hypochlorite was

least effective, producing <1 log_{10} reduction in infectivity. Statistical analysis of germicide efficacy against MHV using one-way ANOVA showed that the mean log_{10} reductions for the six germicides were significantly different from one another ($p<0.0001$). Further analysis of the mean log_{10} reduction of MHV was done using the Tukey's Multiple Comparison test to determine which germicides produced reductions that were significantly different from each other ($p<0.05$). The results are shown in table 6.4.2. Vesphene IIse (1.33 Log_{10} reduction) did not differ significantly in viral infectivity reduction from chlorine bleach (0.62 log_{10} reduction), Cidex-OPA (1.71 log_{10} reduction), or Clorox Anywhere Spray (1.98 log_{10} reduction). Cidex-OPA did not differ from Purell Hand Gel (2.66 log_{10} reduction) or Clorox Anywhere spray, and Purell Hand Gel did not differ from Clorox Anywhere spray in log_{10} reduction of MHV infectivity.

The same analysis was carried out for disinfection efficacy against TGEV. Infectivity reductions of >3 log_{10} were observed for 70% ethanol (3.19 log_{10} reduction), Purell Hand Gel (4.04 log_{10} reduction), and Clorox Anywhere spray (3.57 log_{10} reduction). TGEV exposures to Vesphene and Cidex produced intermediate TGEV infectivity reductions of 2.03 and 2.27 log_{10}, respectively. As seen with MHV, hypochlorite exposure resulted in <1 log_{10} reduction in infectious TGEV titer (0.35 log_{10} reduction). Statistical analysis using one-way ANOVA showed that the mean log_{10} reductions were significantly different ($p<0.0001$) among the 6 germicides. Tukey's Multiple Comparison test on reductions of virus infectivity showed that Cidex-OPA (2.27 log_{10} reduction) did not differ significantly from Vesphene IIse (2.03 log_{10} reduction) or 70% ethanol (3.19 log_{10} reduction) ($p>0.05$), and 70%

ethanol did not differ significantly from Purell Hand Gel (4.04 $\log_{10}$ reduction) or Clorox Anywhere Spray (3.51 $\log_{10}$ reduction) ($p>0.05$). Similarly, Purell Hand Gel (4.04 $\log_{10}$ reduction) and Clorox Anywhere Spray (3.51 $\log_{10}$ reduction) did not differ significantly ($p>0.05$) in virus infectivity titer reduction.

Two-way ANOVA

A two-way ANOVA was used to compare two independent variables, germicide and virus type. This test aids in determining how much of the variability in $\log_{10}$ reduction is explained by each of these two independent variables and their interaction. As a whole, the infectivity reduction results are statistically significant ($p<0.0001$). A Type III Sum of Squares test determined variation among mean reduction results. The type of germicide used ($F = 39.75$, $p < 0.0001$) had a greater influence on viral reduction than did the virus tested ($F = 12.40$, $p = 0.0008$). Additionally, the ANOVA test evaluated the interaction between germicide and virus, and it was determined that there is a statistically significant interaction between virus type and germicide type ($F = 6.00$, $p = 0.0001$).

Table 6.4.1 Disinfection Efficacy Against MHV and TGEV on Carrier Surfaces

	Mean Log10 Reduction in Infectivity (95% CI)	
Germicide	**MHV**	**TGEV**
Chlorine bleach	0.62 (0.52, 0.72)	0.35 (0.24, 0.45)
Vesphene IIse	1.33 (1.16, 1.51)	2.03 (0.89, 3.17)
Cidex-OPA	1.71 (1.35, 20.7)	2.27 (2.09, 2.45)
70% Ethanol	3.92 (3.32, 4.53)	3.19 (2.97, 3.40)
Purell Hand Sanitizer	2.66 (1.77, 3.56)	4.04 (3.57, 4.51)
Clorox Anywhere Hand Sanitizing Spray	1.98 (1.68, 2.27)	3.51 (3.29, 3.73)

Table 6.4.2 Tukey's Multiple Comparison Test for Statistical Difference between Germicides.

Tukey's Multiple Comparison Test	MHV Significant? P < 0.05?	TGEV Significant? P < 0.05?
Bleach vs Vesphene	No	Yes
Bleach vs Cidex	Yes	Yes
Bleach vs 70% Ethanol	Yes	Yes
Bleach vs Purell	Yes	Yes
Bleach vs Clorox Spray	Yes	Yes
Vesphene vs Cidex	No	No
Vesphene vs 70% Ethanol	Yes	Yes
Vesphene vs Purell	Yes	Yes
Vesphene vs Clorox Spray	No	Yes
Cidex vs 70% Ethanol	Yes	No
Cidex vs Purell	No	Yes
Cidex vs Clorox Spray	No	Yes
70% Ethanol vs Purell	Yes	No
70% Ethanol vs Clorox Spray	Yes	No
Purell vs Clorox Spray	No	No

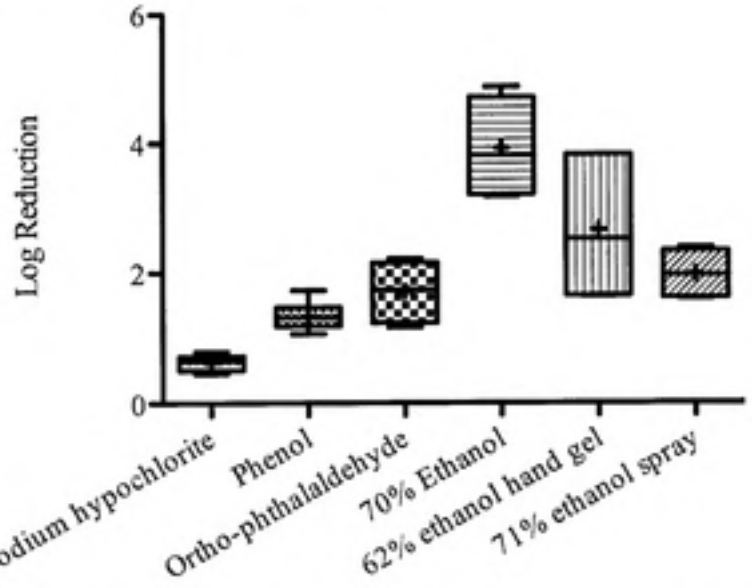
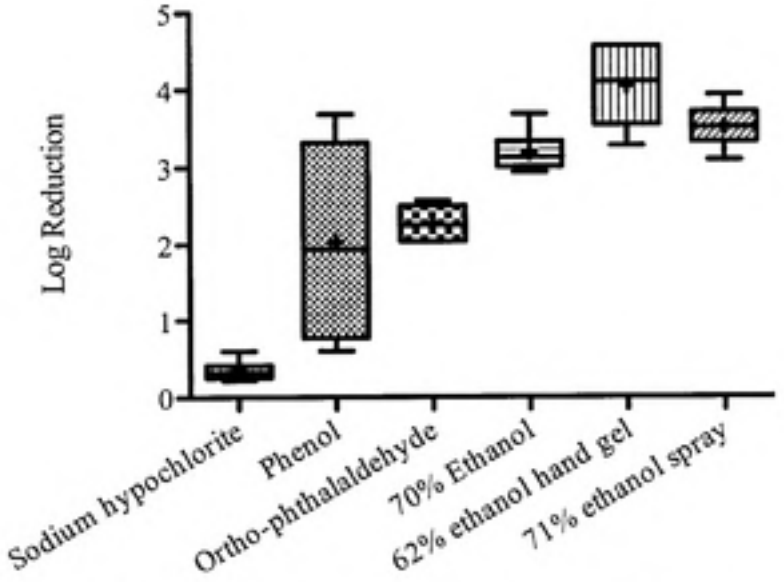


Figure 6.4.1 Mean $\log_{10}$ reduction of MHV viral titer after exposure to germicide.



6.4.2 Mean $\log_{10}$ reduction of TGEV viral titer after exposure to germicide.

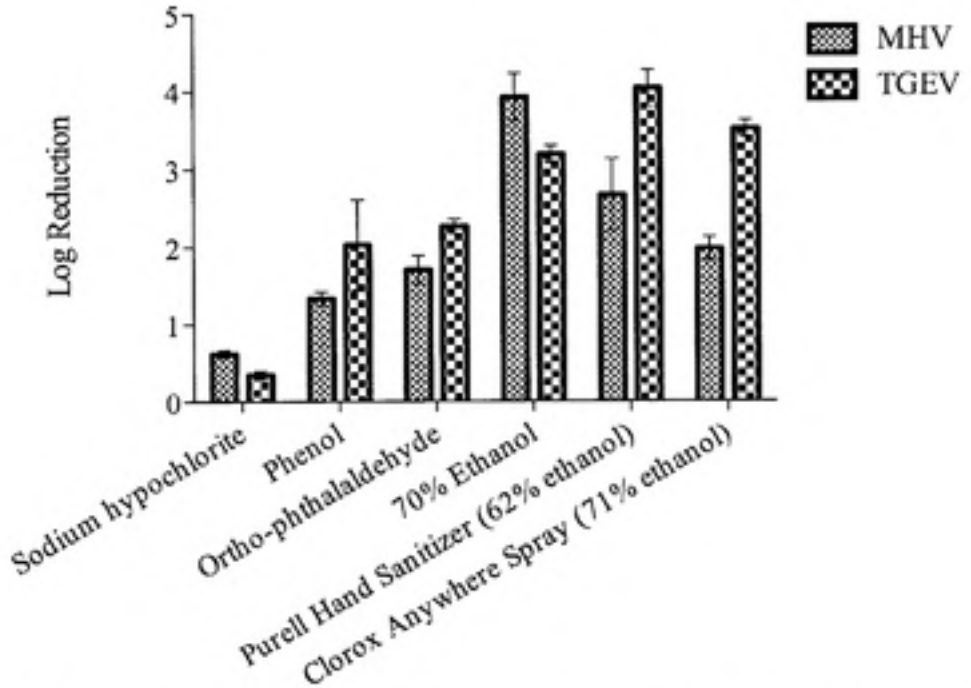


Figure 6.4.3 Comparison of mean $\log_{10}$ reduction between MHV and TGEV.

7. DISCUSSION

7.1 Cytotoxicity and Infectivity Interference of Germicides and their Neutralizers for Cell Cultures

Testing for cytotoxicity on DBT and ST cell lines was done to determine if germicide chemicals and neutralizing solutions caused cytotoxic effects to the cell culture monolayers that might alter virus attachment to and infection of these cells. Because plaque and MPN assays were performed to determine virus infectivity titer, it is desirable that there be no positive or negative effects on the cell culture monolayer when exposed to germicides, neutralizers, or a combination of the two. Test chemicals displaying no observable cytotoxic effect to the cell monolayers were subjected to virus infectivity interference testing. Sattar et al. (2003) recommended testing the germicides for cytotoxicity at use-dilution, 1:20 dilution, and an additional 1:10 dilution. In this study cytotoxicity and virus infectivity interference tests focused on use the use-dilutions of the germicides. Generally, no substantial difference in cytotoxic effects was observed when testing both a use dilution and a further 1:10 dilution of the germicide.

The exposure condition of greatest importance for adverse effects on cell cultures and infectivity assays was the germicide-neutralizer combination. During the carrier-based disinfection testing protocol, the cell monolayers were exposed to an eluent containing germicide, a neutralizing solution, and beef extract elution solution. Lysol germicide spray, in combination with 3% glycine, caused cytotoxic effects after 1 hour of incubation and exposure. Previous studies used 3% glycine to neutralize

the quaternary ammonium compound, however, the cell lines used in each study differed (Rutala, Peacock et al. 2006). Variation in test viruses and corresponding host cell lines will influence cytotoxic outcomes because of varying susceptibility of the host cell line to chemical exposures.

Cidex-OPA was tested in combination with 5% glycine because exposure to Cidex-OPA and 3% glycine produced significantly greater cytotoxic effects after 24 hours of incubation when compared to other chemical combinations. Additionally, Cidex-OPA likely had fixative properties. This was evident in the 1:10 dilution of germicide, where greater damage was observed than in the use-dilution (undiluted) of germicide wells.

All of the remaining germicide-neutralizer solutions produced no observable cytotoxic effects after 1 hour of incubation, so these combinations were then subjected to virus infectivity interference testing.

7.2 Testing for Virus Elution Efficiency

These experiments showed a loss of initially applied viral titer ranging from 0.10 to 0.74$\log_{10}$ when dried virus was eluted from carriers after chemical neutralization. These losses of virus infectivity from the elution process are consistent with previous findings for virus elution from stainless steel using beef extract eluting solution. For surface disinfection of viruses, a reduction in virus infectivity of 2-4$\log_{10}$ has been proposed as an acceptable level of performance (Bellamy 1995; Rutala, Barbee et al. 2000; Steinmann 2001; Sattar, Springthorpe et al. 2003). The MHV and TGEV virus stocks had titers of approximately 10^7 plaque forming units (PFU)/mL, so the 20µL of stock virus dried onto the stainless steel carriers contained

approximately 10^5PFU/μL. Therefore, even with ~0.5 $\log_{10}$ loss of infectivity titer during elution, a 4 $\log_{10}$ reduction in viral infectivity by the test germicides could still be observed. A loss of 0.45$\log_{10}$ to the carrier surface still allowed for the observation of as much as a 4$\log_{10}$ reduction in virus infectivity titer. It should be noted that there has been little work reported that attempted to determine the status and fate of virus particles not removed from the carrier surface by elution (Yilmaz and Kaleta 2003).

7.3 Testing for Interference with Virus Infectivity Assay

Preventing cytotoxicity from residual germicide in virus samples is vital for accurate assessment of virucidal effectiveness of germicides. Damage to the cell culture monolayers from residual germicide in samples or from germicide/chemical neutralizer combinations may not be visible by microscopic examination, but may result in alteration of the ability of the virus to successfully infect the host cell. Such alteration of infectivity could be by interference with viral attachment to cells or subsequent steps in the process of virus infection and replication. Cell culture monolayers that appear healthy but have altered susceptibility to infectious viruses may be unable to support successful viral replication, (Sattar, Springthorpe et al. 2003) resulting in underestimation of viral titers and overestimation of germicide efficacy.

An overlap of the 95% CI of experimental and control assays indicates that the viral titer quantified by these assays does not differ significantly. Differences may be accounted for by the variation of viral particles present in the 20μL of

inoculum. This variability is further accounted for by using three replicates of experimental and control assays per trial.

It is also important to achieve complete neutralization of germicide activity to ensure that all exposure trials have a standard contact time of 1 minute. Using the correct neutralization solution is essential in stopping virucidal activity (McDonnell, Klein et al. 1998) at the desired contact time. Neutralization can occur by adding a neutralizing chemical or by dilution of germicide in another liquid medium (Sattar, Springthorpe et al. 2003). Incomplete neutralization of the chemical germicide is a primary source of cytotoxicity and other cellular level effects on susceptibility to virus infection.

Three germicides yielded viral titers on exposed cell layers that were significantly greater than the titer on control cell layers. The phenolic germicide and two ethanol-based germicides (62% and 71% ethyl alcohol) increased the number of countable plaque forming units on DBT and ST cell lines. This suggests that the germicide-neutralizer mixtures may make the DBT cells more susceptible to infection by MHV. It is also possible that the small sample size (n=3) introduces statistical variation. An increase in the viral titer due to exposure of the virus to the chemical and then exposure of host cells to the neutralized and eluted chemical mixture may result in an underestimation of virucidal activity for these chemicals.

In previous studies, Letheen broth was used to neutralize quaternary ammonium solutions (Rutala, Barbee et al. 2000). Another virus disinfection study applied 3% glycine to quaternary ammonium-based germicides to stop virucidal activity (Rutala, Peacock et al. 2006). These studies used different host cell lines

(Buffalo green monkey kidney and A549 cells) while this study utilized DBT and ST cell lines. In this study, 3% glycine was successful in neutralizing the ethanol and *ortho*-phthalaldehyde chemicals on both the cell lines used to assay the infectivity of MHV and TGEV. This finding supports previous evidence of the efficacy of 3% glycine as a universal neutralizing solution for chemicals without having to search for individually tailored chemical neutralizers. However, proper cytotoxicity/virus infectivity interference testing should be performed with each chemical and on each host cell line used for viral propagation and assay.

7.4 Testing for Germicide Efficacy Against MHV and TGEV on Carrier Surfaces

The efficacy of six hospital surface germicides was tested against two coronaviruses, MHV and TGEV, used as surrogates for SARS-CoV. Based on previous studies, a 3 $\log_{10}$ reduction was chosen as the benchmark for effective virucidal activity against coronaviruses on surfaces (Abad, Pinto et al. 1997; Rutala, Barbee et al. 2000; Sehulster and Chinn 2003; Sattar 2004).

For MHV, the three ethanol-based germicides (71%, 70%, and 62% ethanol) gave the greatest reduction in infectivity ranging from 1.5 to 4.9 $\log_{10}$. This was greater than the reductions observed with hypochlorite (0.62 $\log_{10}$ reduction), phenolic (1.33 $\log_{10}$ reduction), and orthophthalaldehyde (1.71 $\log_{10}$ reduction) germicides. These same trends are evident in tests of these germicides against TGEV. The ethanol-based germicides gave infectivity reductions ranging from 2.9 to 4.6 $\log_{10}$. The reductions observed with hypochlorite, phenolic, and orthophthalaldehyde germicides were, 0.35 $\log_{10}$, 2.03 $\log_{10}$, and 2.27 $\log_{10}$, respectively. Overall, the phenolic and orthophthalaldehyde germicides had greater

virucidal activity against TGEV (2.03 To 2.27 $\log_{10}$ reduction) than against MHV (1.33 to 1.71 $\log_{10}$) reduction. However, only the reductions in infectivity by the orthophthalaldehyde statistically significantly differed between MHV and TGEV ($p = 0.0203$).

Several previous studies have determined that surface disinfection is essential in preventing viral transmission from surfaces to humans (Rutala, Barbee et al. 2000). The risk of acquiring infection decreases proportionately to the amount of viral agent present in surfaces(Sattar 2004). Proper use of virucidal chemicals can decrease the risk of exposure and transmission of infection by reducing numbers of virus present on a surface. This study shows that ethanol-based germicides achieve the greatest reduction in viral titer on surfaces. These findings are consistent with previous disinfection studies. Sattar, et al. (1989) found that 70% ethanol applied to human coronavirus 229E dried onto a stainless steel surface reduced viral titers by >99.9%. However, Rutala et al. (2006) performed a study on the efficacy of hospital germicides against Adenovirus 8 using the same carrier-based method and produced slightly differing results. The Rutala et al. study included three of the same germicides tested in this study: Cidex OPA (orthophthalaldehyde), Vesphene IIse (phenolic), and 70% ethanol. The results of that study showed 4.37 $\log_{10}$ reduction using Cidex OPA and 0.41 $\log_{10}$ reduction using Vesphene IIse while this study determined that Cidex OPA and Vesphene IIse produced similar results on each cell line. Cidex OPA produced 1.71 $\log_{10}$ reductions (MHV) and 2.27 $\log_{10}$ reductions (TGEV), and Vesphene IIse produced 1.33 $\log_{10}$ reductions (MHV) and 2.03 $\log_{10}$ reductions (TGEV). Rutala et al. (2006) also tested 70% ethanol, for which similar

results were produced compared to this study (1.97 to 4.62 $\log_{10}$ reduction and 1.5 to 4.9 $\log_{10}$ reduction, respectively). Coronaviruses are enveloped viruses while adenoviruses are non-enveloped, which may explain some of the differences in disinfection results.

Hypochlorite demonstrated <1 $\log_{10}$ of reduction for both viruses when applied at the use-dilution prescribed by the manufacturer (1:100). These results are also consistent with previous coronavirus disinfection studies with hypochlorite. A sodium hypochlorite solution (10mg/L) required 30 minutes of exposure to produce 100% kill of SARS-CoV suspended in water samples (Wang, Li et al. 2005). Additionally, the Wang (2005) study reported an inactivation rate of only 44% after a 1-minute contact time in solution. Sattar et al. (1989) reported 99.9% reduction of viral titer for human coronavirus 229E when 0.10% and 0.50% sodium hypochlorite solutions were tested with one-minute contact times. The results of the present study suggest that the 1:100 use dilution recommended by the manufacturer should not be recommended for use on surfaces with suspected contamination by coronaviruses. This is because <1 log10 inactivation was obtained after 1 minute of exposure of coronaviruses dried onto a stainless steel surface. Increases in both sodium hypochlorite concentration and contact time should be evaluated to determine whether these factors would improve virucidal activity to >3 $\log_{10}$ performance target.

The chlorine bleach solution was created by diluting 1mL of a 6% hypochlorite solution with 99mL of hard water for a final concentration of 600 mg/L hypochlorite. This concentration produced a <1 $\log_{10}$ reduction of MHV and TGEV. The US Environmental Protection Agency National Primary Drinking Water

Regulations (1998) recommends the use of 3-4mg/L of free chlorine be used to disinfect drinking water. The discrepancy between the hypochlorite concentrations necessary for effective disinfection of viruses in drinking water that that required for disinfection of these viruses dried onto surfaces may be explained by the high chlorine demand of the matrix in which the viruses were suspended. Viruses were contained in a matrix of cell culture medium, which contains a high level of proteins that create chlorine demand. Proteins can undergo irreversible reactions with hypochlorite ions, rendering the hypochlorite unavailable for viral inactivation. When 20µL of virus stock solution was placed on the steel carrier, it was dried for two hours, evaporating the liquid but leaving behind the proteins in the cell culture matrix and the virus particles. This dried material has the potential to exert chlorine demand. One of the objectives of this research was to test hospital germicides, as they would be used in field conditions. Suspending test viruses in a proteinaceous matrix simulates the conditions under which viruses shed by human hosts occur in healthcare environments. Viruses are not shed an infected host as single purified particles; they occur in feces, mucus, and other proteinaceous matrices. Rutala et al. (2006) used 1:10 and 1:50 dilutions of Clorox bleach and found 4.87 and 1.99 $\log_{10}$ reductions, respectively. These results suggest that changing the recommended use-dilution of chlorine bleach may address the problem of chlorine demand and result in effective inactivation of coronaviruses shed from human hosts.

For viruses dried on stainless steel surfaces, Cidex-OPA exposure of 1 minute resulted in 1.5-2.2$\log_{10}$ reduction in infectivity of MHV and 2-2.5$\log_{10}$ reduction of infectivity of TGEV. Vesphene IIse produced a mean 1.3 $\log_{10}$ reduction of MHV

and 2.0 $\log_{10}$ reduction of TGEV. The 'Technical Information' report on Cidex-OPA (Johnson&Johnson Company) states that the solution passed the virucidal efficacy test outlined by the EPA (2005) on a human coronavirus. This protocol requires a 3-4 $\log_{10}$ reduction in viral titer. Additionally, the test was performed with a 5-minute exposure period. Vesphene IIse also passed the EPA virucidal assay method when tested against a coronavirus (Avian infectious bronchitis) using a 10-minute exposure period (STERIS-Corporation 2004). This current study performed with coronaviruses MHV and TGEV used only a 1-minute exposure period, suggesting that virucidal efficacy is compromised if these products are not used strictly according to label directions and contact time is short.

When log10 reductions of the different germicides were subjected to statistical analysis, they were statistically significantly different ($p < 0.0001$) based on a two-way ANOVA test. These results indicate that the mean $\log_{10}$ reduction differed significantly among the six germicides. Viral reductions differed significantly based on both the type of germicide and the type of virus tested. The amount of variation among means was examined and germicide ($F = 39.75$) has a more significant effect on viral reduction than did virus type ($F = 12.40$). The interaction term ($p = 0.0001$) evaluates whether the difference between germicides is the same for both viruses, or, similarly, whether the difference between viruses is the same for each germicide. P-values were <0.05, indicating that reduction of viral titer was significantly different for germicide and for virus as independent variables. Hence, the selection of germicide and virus will each influence the resultant magnitude of reduction of virus infectivity titer.

Surrogate viruses are necessary for studying SARS-CoV disinfection because the highly infectious nature of the virus and the level of containment required make work with this virus difficult (Steinmann 2004). MHV and TGEV represent two of the groups in the *Coronaviridae* family that contain other human coronaviruses. Both viruses are easily propagated in cell culture and can be assayed for infectivity by plaque and quantal MPN assays (Hirano, Fujiwara et al. 1974); (McClurkin and Norman 1966). Greater infectivity reductions were observed with TGEV than with MHV on surfaces, indicating that TGEV may be less resistant than MHV to inactivation by germicides. To better estimate the efficacy of germicides against SARS-CoV, studies should use the more resistant surrogate coronavirus as a model and benchmark for performance. A virus with greater resistance to germicide chemicals can serve as conservative surrogate for modeling risk in worst-case scenarios.

Many of the previous disinfection studies used suspension methods for testing germicide efficacy (Rutala, Barbee et al. 2000; Rabenau, Kampf et al. 2005; Wang, Li et al. 2005). These studies reported generally greater efficacy against viruses than studies performed using the carrier method. Viruses may be more resistant on surfaces than in suspension because they can adsorb to the surface or become embedded in organic material (Sattar, Springthorpe et al. 2003). Virus dried onto a surface may be more difficult to fully expose to and inactivate with chemical germicides than viruses suspended in liquid. Thus, it is possible that suspension tests overestimate the level of antimicrobial activity of viruses on surfaces. Carrier-based methods may more closely resemble real environmental conditions where viruses

contaminate surfaces, and provide a more conservative estimate of germicide efficacy activity against viruses that have become dried onto environmental surfaces.

8. CONCLUSION

This study aimed to evaluate eight healthcare surface germicides for their virucidal capabilities against two coronaviruses. By testing chemicals at their manufacturer-recommended use-dilution and drying surrogate viruses onto stainless steel surfaces, this study attempted to recreate the conditions under which healthcare facilities want to control SARS-CoV.

- Level of effectiveness varied based on active ingredient. Generally, the ethanol-based germicides were most effective in reducing viral titer of dried virus. 70% ethanol produced >3 $\log_{10}$ reduction of MHV and TGEV. The products containing 62% and 71% ethanol reduced infectivity of MHV and TGEV by 2-4 $\log_{10}$. These chemicals are considered effective at reducing viral presence on environmental surfaces. The phenolic- and othophthalaldehyde-based germicides reduced MHV and TGEV infectivity by 1-2 $\log_{10}$. These chemicals are considered somewhat effective at reducing viral titer. 1:100 Chlorine bleach (sodium hypochlorite) was ineffective at reducing the infectivity of MHV or TGEV with <1 $\log_{10}$ reduction.
- Several chemical germicides were determined less effective or ineffective in reducing infectivity. Further study is recommended in order to determine use-dilutions that will effectively inactivate SARS-CoV on surfaces. The use-dilution for chlorine bleach is 1:100; however, this concentration did not inactivate the

surrogate coronaviruses. Recommendations for chlorine bleach, Cidex-OPA, and Vesphene IIse may need to be increased to improve the mean $\log_{10}$ reduction of viral titer.

- The level of virucidal activity was significantly different between surrogate viruses, MHV and TGEV. This means that some germicides were more effective against one virus than the other. Generally, a greater $\log_{10}$ reduction was observed against TGEV. This may suggest that MHV is a better surrogate for SARS-CoV research because it is more difficult to disinfect and reduces the likelihood of overestimating effectiveness.
- This study was unsuccessful at properly neutralizing two germicides: T.B.Q. and Lysol® Disinfectant Spray. The active ingredient in these solutions is a quaternary ammonium compound (QAC). While previous disinfection studies used 3% glycine or Letheen broth to neutralize QACs, the combination of QACs with these neutralizing solutions produced cytotoxic effects to delayed brain tumor and swine testicular cell lines. Identification of a proper neutralizing solution is necessary before these germicides can be tested for virucidal activity.
- Future research on this subject should be performed to evaluate the method for efficacy testing and to follow up with the effectiveness of the tested germicides against SARS-CoV. Future research should determine the role that drying time has on viral titer. Additionally, the reduction of infectivity should be compared when using the suspension method and carrier method simultaneously. This concurrent test will allow for the comparison of both methods to determine whether there is a significant difference between the two. Finally, this study

should be repeated using SARS-CoV to determine if the two surrogate viruses, MHV and TGEV, are suitable models for the response of SARS to these germicides.

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