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The Antiviral Action of Common Household Disinfectants and Antiseptics Against Murine Hepatitis Virus

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THE ANTIVIRAL ACTION OF COMMON HOUSEHOLD DISINFECTANTS AND ANTISEPTICS AGAINST MURINE HEPATITIS VIRUS

by

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A thesis

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Abstract

The 2003 outbreak of Severe Acute Respiratory Syndrome (SARS) infected over 8,000 people and killed 784 leaving many questions concerning the effectiveness of common household disinfectants and antiseptics at preventing viral transmission. In order to determine how well standard disinfectants and detergents were at eliminating viral particles from surfaces, the antiviral action of triclosan, pine oil, bleach, chloroxylenol and quaternary ammonium compound/ethanol based products were assayed against Murine Hepatitis Virus (MHV), a virus genetically similar to SARS. Using the Environmental Protection Agency (EPA) guidelines for the virucidal assay, it was determined that alkyl dimethyl benzyl ammonium saccharinate in 79% ethanol (Lysol Disinfectant Spray®), chloroxylenol (Dettol Liquid Antiseptic -Disinfectant®), sodium hypochlorite (household bleach), triclosan (Clean & Smooth Soap®) and pine oil (Pine-Sol®) were effective against MHV when used at the recommended concentrations. Products were then diluted outside of the recommended range and were assayed to determine the efficacy of these products when used incorrectly. Three of these products, alkyl dimethyl benzyl ammonium saccharinate in 79% ethanol (Lysol Disinfectant Spray®), triclosan (Clean & Smooth Soap®), and sodium hypochlorite (household bleach), when used incorrectly, did not demonstrate complete inactivation of MHV and the presence of MHV infection was detectable after product treatment.

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Introduction

The Severe Acute Respiratory Syndrome (SARS) virus infected 8098 people and killed 774 in 2003 (1). Since SARS was a new emerging disease there was no known medical treatment or vaccine available to prevent further cases or to help the infected survive. The only means of containing the viral outbreak was to practice isolation, infection control and follow the etiology of the virus, practices that have been performed for centuries (2). SARS was discovered in March of 2003 and shortly after was classified as a member of the family of viruses known as *Coronaviridae* (3). The emergence of the virus is believed to have begun in the Guangdong province of China in November of 2002 (1, 4, 5, and 6). Many techniques were used to discover the etiological agent of the SARS virus including cell culture, RT-PCR, electron microscopy, histopathological examination and serological analysis (5 and 7). The focus of the search was largely on respiratory agents and those viruses that target the lower respiratory tract (5). In an attempt to isolate the virus, successful infection was achieved using the Vero E6 cell line and within 24 – 48 hours the viral cytopathogenic effect was seen (4, 5). Electron microscopy revealed a distinct ring formed by the helical nucleoplasmid and spikes on the viral envelope, both of these attributes distinct to Coronaviruses (2).

Because of its enveloped outer membrane and viral RNA, it was initially believed that SARS most resembled the viruses from Group II of the *Coronaviridae* (2). Although, recent phylogenetic analyses has suggested that SARS is equally distantly related to any of the groups and belongs in a new group, Group IV (8 and 9). SARS and Murine Hepatitis Virus (MHV) share many structural and genetic similarities. For example, both viruses contain two overlapping Open Reading Frames (ORF) known as

ORF 1a and ORF 1b, which are important to initiate translation (10 and 11). Both viruses also show similar genome organization (11). However, there are also differences between the two viruses including the replicase gene which, in MHV, encodes three proteinases and in SARS only encodes two proteinases. These proteinases are important for the cleavage of polyproteins into mature proteins which are necessary for virus transcription (10). Another critical difference is that while SARS is a bio safety level (BSL)-3 agent, MHV can be studied in a BSL-2 facility. Thus, analysis of MHV may serve to answer questions about SARS more quickly and without the need to set up complex research facilities.

Coronaviruses

Coronaviruses are from the order Nidovirales and the family *Coronaviridae*. They are large, enveloped, positive stranded RNA viruses ranging from 27 to 32 kb (4, 8 and 11 - 14). *Coronaviridae* consist of two genera: Coronaviruses and Toroviruses. They differ by their virion morphology, the genome length and also, the Toroviruses only infect animals (12 and 14). Coronaviruses, for the most part, cause respiratory and enteric diseases in humans and in animals (4). They have the largest genome known of all the RNA viruses and they also share a very high recombination frequency (12). The name corona (Latin for crown) was attributed to Coronaviruses because their envelope is studded with long spikes which look like a crown (12).

There are three different serological groups in the *Coronaviridae*, antigenic groups I, II and III. They were first separated based on serological testing and now they are separated based on similar genomic sequences, host range, antigenic relationships and

the organization of the genome (4). The first two groups contain mammalian viruses and the third group only contains avian viruses as listed below (4).

Antigenic group I: *HCoV 229E, Human Coronavirus -229E; TGEV, Porcine transmissible gastroenteritis virus; PRCoV, porcine respiratory Coronavirus; CCoV, canine Coronavirus; FIPV, feline infectious peritonitits virus; FECov, feline enteric Coronavirus; RBCoV, rabbit Coronavirus*

Antigenic group II: *MHV, mouse hepatitis virus; HCoV-OC43, Human Coronavirus – OC43; HEV, porcine hemagglutinating encephalomyelitis; TCoV, turkey Coronavirus; SDAV, sialodacryoadenitis virus; BCoV, bovine Coronavirus*

Antigenic group III: *IBV, avian infectious bronchitis virus; TCoV, turkey Coronavirus*

In figure 1, some of the Coronaviruses are illustrated in a phylogenetic tree to better illustrate how closely related the different Coronaviruses are and the different antigenic groups there are. A DNA microarray was designed to determine if this novel virus was associated to an existing viral family. The strongest results yielding the best hybridizing elements were to the *Coronaviridae* and the *Astroviridae* (3). These results were further analyzed to determine the genetic identity to the existing viruses in that family. A BLAST analysis using 157 amino acids of the nucleocapsid resulted in a 33 % identity to MHV (3). In animals, Coronaviruses can lead to virulent respiratory, enteric, hepatitis and neurological diseases also some types of Coronaviruses can cause severe systemic diseases resulting in death (2). In humans, Groups 1 and 2 used to be associated with mild respiratory illnesses until SARS (15 and 16). About 30% of all upper respiratory illnesses is caused by two types of Human Coronaviruses, HCoV-229E found in group 1 and HCoV-OC43 found in group 2 (4, 13, 17 and 18). The SARS virus is unlike other

Coronaviruses because of the effect it had on the lower respiratory tract and how it caused disease in more than one host species (17). Data have suggested that the SARS virus is the first virus to be grouped in a new serological group labeled as group IV (19). The structural proteins such as the spike, nucleocapsid, membrane and small membrane proteins seemed to have less sequence conservation as compared to the enzymatic proteins such as helicase and polymerase. This suggests SARS should be classified in its own distinct group (4). These studies also suggest that SARS originated from a non-human host and jumped the species barrier to infect humans (17). But other studies analysis suggest that the virus is most likely a split-off of group II because of genetic similarity that could have occurred by recombination of two human Coronaviruses or an animal and a Human Coronavirus or even mutations in an existing human Coronavirus that led to newly acquired virulence factors (10 and 17). There are other human Coronaviruses not diagrammed below such as HCoV-OC16, HCoV-OC37 and HCoV-OC48 which are known to cause colds in humans but animal models or cell culture cannot be utilized as methods to study these viruses and therefore a method does not exist for studying these viruses (20).

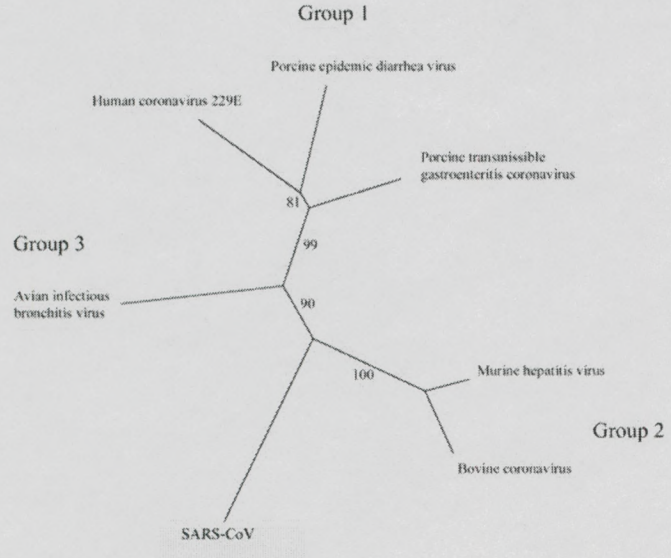


Figure 1. Phylogenetic tree of different Coronaviruses (2).

Structure of Coronaviruses

Coronaviruses have five different structural proteins to aid with the formation of the virion (12). The spikes on the surface of Coronaviruses are formed by the spike (S) glycoprotein. This formation leads to a distinct crown which is an attribute to the *Coronaviridae*. The S protein is further divided into three different groups, external domain, transmembrane domain and a carboxylterminal cytoplasmic domain (12). The external spike glycoproteins, S1 and S2, are involved with the formation of the spike. The S1 protein is involved with the structure of the globular portion and the S2 protein forms the rod below the globular portion. The S1 protein mutates frequently and therefore is believed not to have a conserved sequence whereas the S2 protein is believed to be more of a conserved structure because of the lack of mutations (12). The S1 protein is responsible for binding to the specific receptors on other cells. Due to the fact that the sequences are not conserved in the S1 protein and deletions and mutations are common allows for the protein to alter the antigenicity and the pathogenicity of the virus (12). During virus maturation for MHV the S protein is cleaved and the S1 and S2 remain as the spike but non-covalently bound. It is believed that this cleavage allows for improved cell fusion and viral infectivity (12). Some of the viruses in the antigenic group I do not allow for the cleavage of the S protein which means the cell fusion and viral infectivity occur not as efficiently.

The hemagglutinin-esterase (HE) glycoprotein form smaller spikes on the surface of the viral particles (12). They are not present in the viruses from the *Coronaviridae* antigenic group I and are only present in some of the group II and group III viruses. The HE protein is believed to have been introduced into the Coronaviruses by

recombination with the mRNA encoding for the HE of influenza C (12 and 17). There is about a 30% sequence similarity between the Coronaviruses and Influenza C viruses HE protein (12). When present, the HE protein is involved in hemagglutinin and hemadsorption which allows for the virus to adhere to the red blood cells and cause the cells to agglutinate. It is believed that the HE protein might also have involvement in viral entry and release. The HE protein in some of the viruses from group II along with the presence of the S protein has demonstrated initial binding of the virus to the host cell. Serial passaging of these group II viruses that contain the HE protein can lead to the deletion of the HE protein, concluding that this structure is not critical for virus infection (12).

The membrane (M) glycoprotein is associated with the envelope of the virus and viral assembly and is involved with determining the budding site of the virus (12). Only a small portion of the aminoterminal domain of the M protein can be found exposed outside of the viral envelope or in some viruses like MHV this protein can also be located internally in what is known as the internal core (12). The internal core is the structure that surrounds the RNA genomic material.

The small membrane (E) protein is also associated with the envelope and with the assistance of the M glycoprotein determines the budding site of the viral particle (12). The E protein can always be found next to the M glycoprotein on the envelope of the viral structure but the M glycoprotein can be located next to the E protein or the HE glycoprotein. The M glycoprotein and the E protein are both required for viral assembly.

Located inside of the internal core the nucleocapsid (N) phosphoprotein interacts with the RNA genomic material (12). This interaction of the N protein with the viral

RNA genomic material allows for further interaction with the M protein and ultimately the formation of viral particles (12). The N protein is known to have three conserved domain regions, one of them being a domain that is responsible for the RNA binding (12).

RNA Recombination

There is a very high frequency rate of RNA recombination for Coronaviruses. Throughout the entire *Coronaviridae* it was estimated that the frequency of recombination is as high as 25% (12 and 14). MHV was the first Coronavirus that demonstrated such a high rate of RNA recombination but now two other viruses have been added to the list and they can also demonstrate this recombination in both *in vitro* and *in vivo* (12). Due to this fact it is believed that development of a vaccine would not be beneficial to the public and also very difficult to make an effective vaccine because of the high frequency of the RNA recombination rate.

Coronavirus RNA synthesis involves a unique mechanism which allows discontinuous RNA transcription and polymerase jumping known as the copy-choice mechanism (12). The viral polymerase on an incomplete RNA template at some random point switches to a different homologous RNA template and continues with the RNA synthesis in this manner until complete (12). The RNA dependent RNA polymerase can also generate point mutations, large deletions and even insertions of foreign RNA into the genome and is known to be error prone (17).

RNA recombination is fundamental for the evolution of Coronaviruses. It is believed that this is the reason for such a high RNA recombination rate. Recombination events have led to new IBV strains by the natural association of poultry flocks with

different viral strains (12). It is believed that the high frequency rate of RNA recombination is one of the major contributing factors that can or has evolved into different Coronaviruses never seen before.

Survival

Coronaviruses in general can survive for 21 days from 4° C to -80° C with a minimal reduction in concentration and can survive beyond 21 days if the temperature is kept at -80°C throughout this time period (2). Specifically HCoV-229E infectivity was studied to still be possible after 3 hours of being dried on surfaces such as aluminum, latex gloves and sterile sponges (13). This same study shows the HCoV-OC43 can only survive 1 hour or less on the same surface (13). SARS can only survive up to 48 hours in hospital wastewater, sewage or dechlorinated tap water (21). In feces from SARS patients with diarrhea the virus can last up to 3 days (21). If the temperatures were kept in the colder range than the SARS virus can persist up to 17 days in feces. The fact that SARS and other Coronaviruses can endure for long periods of time allows for transmission to occur successfully and disinfection of contaminated surfaces should be taken seriously.

The Signs and Symptoms

The signs and symptoms of the SARS virus include $\geq$ 38° C (>100.4° F) temperature, lower respiratory tract problems such as coughing, difficulty breathing, and a dry cough, traveling to an area which has been noted for transmission of the disease, or contact with an individual that has been diagnosed or is believed to have been exposed to the SARS virus (22).

SARS cases were classified into three different groups. One group was the suspect case group which does not fit the classic symptoms of the disease, a probable

case group fits the classic definition of the symptoms of the disease, and a discarded case group does not have any pathologically evidence. Cases were then diagnosed and confirmed by laboratory tests such as viral culture, antibody tests, or PCR test. If the results were negative they were tested 28 days later to confirm the finding (2).

Epidemiology

SARS appears to have first been noticed in the Guangdong Region of China during November 2002 (2). From there it is believed to have taken over three months to reach neighboring towns. This is an attribute to a disease with a very low infectious rate. However, during the peak of the virus in May of 2003, 200 new cases were being reported each day. The infectious rate of influenza almost 100 years ago compared to the SARS outbreak traveled the globe faster. SARS is believed to have been taken out of the Guangdong region by a doctor visiting Hong Kong for one night on Feb 21, 2003 at the Hotel Metropole. This occurrence initiated the outbreak. It is believed that the origin of SARS or a relative of this virus originated from animals and was transmitted to humans. The Himalayan palm civets and raccoon dogs are indigenous animals to the Guangdong Province in China and data suggest that these animals might be the origin of SARS (1, 23 and 24).

Serological assays performed on blood samples taken before 2002 suggest that the SARS like viruses circulated throughout the human population well before the outbreak (19 and 23). In the live animal markets in China SARS-CoV like viruses were isolated from the Himalayan palm civets and raccoon dogs. Serological screening was also performed on animal traders and slaughterers and these results indicated that 40% of the traders and 20% of the slaughterers had an antibody reaction to SARS CoV or SARS

CoV like viruses. Further, there is a close similarity between these two viruses. They only differ by 60 to 80 nucleotides which can attribute to cross reactivity of the antibodies and therefore not permitting the SARS virus to circulate before the 2003 outbreak (19). These numbers are very high within the animal and human population and can be indicative of another outbreak. The SARS CoV and animal SARS like virus as discussed previously are related to the Coronavirus species that causes respiratory and gastrointestinal diseases but some researchers feel they are different enough to be grouped in a new antigenic group number IV (19). It is believed that the Coronavirus jumped the species barrier from animals and infected humans. At some point the virus, probably through genetic deletions or mutations, adapted to the human population and caused an outbreak (20). An evolutionary study was performed on different proteins that make up the SARS virus such as the replicase, spike and nucleocapsid proteins. There is significant data to suggest a mammalian origin for the replicase protein, mammalian-avian origin for the spike protein, and avian origin for the nucleocapsid protein (25). Still the evolution of SARS has not been determined. An outbreak caused by a novel agent from an animal requires extensive exposure and viral evolution to cause animal-to-human transmission that eventually leads to human-to-human transmission. This ideal situation for an outbreak is commonly seen in any areas that have wildlife animal markets and should be closely monitored for animal viruses that could evolve into a pathogenic organism for humans.

The studies of confirmed SARS cases were divided into three groups: early, middle and the late phase groups. The early phase describes the SARS cases that were believed to have been the first cases of SARS in Nov. 2002 from the Guangdong Province of China to the first superspreader. A superspreader event is when transmission

of hundreds of cases can be traced back to the same person. The first superspreader event is believed to have occurred in a hospital in Guangzhou where 130 primary and secondary cases could be traced to one individual. Of the 130 cases, 106 of them were hospital acquired cases. All cases from this point, including when a doctor on Feb 21, 2003 visited Hotel Metropole in Hong Kong, started the middle phase. Any case that was confirmed a SARS case after this point falls into the late phase (2).

Prevention

Controlling the SARS outbreak was successful because of isolation, effective contact tracing, quarantining those that were infected by the virus and global involvement. Transmission of the disease is important for its survival and therefore it is important to stop the transmission from an infected person to a healthy individual. Just as important, there were more enhanced infection control procedures that were followed such as safety measures for healthcare workers, isolating SARS patients and disinfecting contaminated surfaces that also lead to the termination of the SARS outbreak in 2003. Also, determining if the reservoir for the SARS virus was humans or animals was important. A more recent paper suggests that bats are the reservoir source and the palm civet and raccoon dog served as an amplification vessel for the virus (26). This assessment can determine if the virus was circulating in the human population before the outbreak or if the virus was able to jump the species barrier from animals to humans.

In a short time the World Health Organization (WHO) was able to prevent further transmission or keep additional cases very low. Two incubation periods of a total of 20 days were used as an indicator that the correct steps were taken to contain the outbreak and prevent it from spreading any further. Careful surveillance of SARS was

occurring to stop the transmission and is still taking place to prevent such an outbreak from occurring again.

In areas where the SARS outbreak was severe, extreme measures resulted in designating hospitals only for SARS patients, quarantining patients and using the military to reinforce these measures. Also, closing movie theaters or other social public places were just some of the other measures used to limit transmission. In Hong Kong, school was cancelled from March to May 2003 (27). In Singapore, a legislation was passed to implement all of the recommended courses of action to prevent further transmission. An electronic picture camera was placed in homes to ensure those that were placed in quarantine or needed to be isolated did not break this rule. In Taiwan, alone, there were over 150,000 persons quarantined (28). The penalty for violating these rules resulted in almost a $6000 fine and 6 months in prison (2).

In hospital settings, more stringent criteria were followed such as frequent hand washing, wearing gloves, masks and gowns all of the time. The Centers of Disease Control and Prevention (CDC) and WHO always recommend hand hygiene and during the SARS outbreak frequent and careful hand hygiene was strongly insisted among hospital care workers, who were more frequently exposed to SARS. If hands were soiled, soap and water were used initially, and if hands were not visibly soiled an alcohol based product was appropriate for use. In the households of suspected SARS persons it was difficult to provide guidance to the other household members on the best control measures because of the uncertainty of when and how the disease was transmitted.

Currently, vaccines are not available for the SARS virus but there are vaccines for other Coronavirus species, which have been developed for animals (e.g. avian

infectious bronchitis virus) (2 and 17). There are many problems developing a SARS vaccine several of which were encountered while developing a vaccine for some of the other Coronaviruses. For Coronaviruses it is difficult to develop a vaccine that provides long term protective immunity and is safe. There is data that some vaccines against certain Coronaviruses lead to disease enhancement (10 and 17). A live, attenuated and recombinant virus vaccine exists for MHV.

While there is not presently a SARS vaccine available, there is a full-length cDNA of the SARS-CoV which provides a template for manipulation of the SARS viral genome for vaccine studies (10). This will allow for the development of a successful vaccine or other therapeutics. Vaccines are developed triggering the spike (S) protein, which will elicit a neutralizing antibody, or other means that affect viral replication (2, 10, 29 and 30). A current study employing the use of a recombinant vaccine modified with the S protein of SARS-CoV and injected into ferrets resulted in a rapid neutralizing effect compared to the controls but also led to inflammatory responses of the liver (31). The benefit of a long lasting immunity that is associated with vaccinations does not seem to be occurring with most types of Coronaviruses. Reinfections can occur, as seen with HCoV-229E, but usually are milder than before (2, 10). The 2003 outbreak of SARS ended due to the old fashioned infection control measures mentioned previously but other therapeutics will be necessary if there ever is another severe outbreak. It has been shown that SARS has a recombinant history leading to different viral strains by host jumping and recombination (32). This is a highly disputed conclusion and others have claimed that either recombination did not occur with SARS or recombination with another virus did not occur.

Transmission

Most Coronaviruses are transmitted through close contact or the inhalation of infected droplets. There were two situations, the Metropole Hotel and the Amoy gardens, that make it unclear during the outbreak if the SARS virus could have spread through airborne transmission. Also, the majority of the SARS outbreaks occurred between households indicating that close person-to-person contact is a means of transmission. A SARS study demonstrated that the amount of RNA virus found in the sputum from SARS patients had an extremely high concentration of the virus indicating that the main route of transmission occurs from the respiratory tract (22).

Hospital care workers and doctors were among those in the high risk groups for becoming infected and represented 21% of probable SARS cases during the outbreak (33). Ill travelers caused SARS to spread to other areas and contributed in bringing others in contact with the infectious disease (34). An example would be the United States of America where only eight confirmed SARS cases were diagnosed with no hospital or community transmission. Why were some areas able to contain the virus and others not? Out of 20 hospitals visited by the mobile SARS containment teams only 25% implemented the correct infection control procedures and contained the virus within the hospital setting which did not allow it to spread to the surrounding communities (1 and 35). Infection rates in countries such as China, Taiwan, and Hong Kong where the containment of the virus was not successful was the result of wide-spread disease transmission (1). Proper infection control measures are one reason why some areas experienced a low transmission rate and also there have been situations where no transmission occurred especially among healthcare workers (36).

SARS to date has been found in three species of animals in the Chinese market (i.e. the masked palm civet cat, the raccoon-dog, and the Chinese ferret badger). These animals are considered delicacies and are sold in Chinese markets. Interestingly enough, antibody tests of those working in the market showed a higher immunity to Coronaviruses than that of the general public (37).

In late February and early March 2003, after several hundred cases of an atypical respiratory illness, which in most cases was diagnosed as pneumonia, Dr. Carlo Urbani of the WHO traveled to China to investigate the matter. He was the first one attributed to recognizing such an unusually high number of cases of atypical pneumonia and alerted everyone of the transmissibility and lethality of this outbreak. In mid-March when similar cases were identified in Canada, Hong Kong and Vietnam the WHO issued a global alert for what was first identified as SARS (4).

The first outbreak of the 21st century heightened awareness of the need to be able to rely on common household products to prevent transmission of emerging pathogens. In order to specifically prove that these disinfectants are efficacious is to choose the right surrogates with which to study. In the US, products must be registered with the EPA in order to claim that they kill germs or are disinfectants against certain organisms. This procedure ensures that US household products have met the disinfectant testing requirements, followed the registration process and the claims on the label of the products are accurate.

During an outbreak the WHO recommends one wash their hands and disinfect contaminated surfaces. This has been a usual practice and suggestion to follow during an outbreak to prevent transmission of the disease (38 - 40). It is extremely rare for viruses

to jump the species barrier from animals to humans but when it does occur it can be catastrophic. As reported by Mary Wilson there are more than 300 diseases that have their reservoir in animals and in time when these diseases jump the species barrier they will develop to new human pathogens (41). It is more likely now then ever for these new pathogens to make their way to the US due to the ease and frequency of human travel and trade.

The characterization of SARS as a novel Coronavirus was determined by comparing the genome to many known respiratory illnesses, the length, the open reading frames and its genome organization were only a few of the determinants used to conclude that the atypical pneumonia cases were in fact a new virus called SARS and the first outbreak of the 21st century (3 and 4).

Test Method

During the SARS outbreak, the recommendation of disinfectants and antiseptics to prevent transmission was one of the major steps for infection control but at the same time the effectiveness of the products against the virus was unknown. The EPA has specific protocols in order to claim a disinfectant has virucidal properties. In order for a product to be registered as efficacious against a certain organism and in this case claim that the product has an anti-viral activity, a method known as a surface test must be followed according to the guidelines of the EPA. As previously described, the surface method involves drying an amount of known virus on a surface and then applying the product to the viral film for a specific contact time. The objective of this study was to assay the effectiveness of common household disinfectant and antiseptic products against

MHV, a virus from group II of the *Coronaviridae*, which in turn can be used as an indicator of how efficacious these disinfectants and antiseptics would be against SARS.

In order to prevent another outbreak of SARS, a better understanding of how well disinfectants prevent viral transmission via surfaces is essential. In order to determine the antiviral activity of these common household products, a surrogate virus was chosen appropriately and an accepted surface efficacy test using guidelines set forth and established by a federal agency, i.e. the Environmental Protection Agency (EPA), was performed. A surface test consists of drying a viral inoculum on a hard non-porous surface and applying the antiviral product to this surface for a designated contact time, neutralization and assessment for infectivity.

Materials and Methods

Cell Lines and Viruses

NCTC clone 1469 (ATCC, Manassas, VA, USA); Mouse liver cells were grown in Dulbecco's Modified Eagle's Medium (ATCC, Manassas, VA, USA) with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose with the addition of 0.6mL/L of gentamicin (Gibco Invitrogen Corporation, Carlsbad, CA, USA) and 10% horse serum (ATCC, Manassas, VA, USA).

BS-C-1 (ATCC, Manassas, VA, USA); African green monkey kidney cells were grown in Eagle's Minimum Essential Medium (Gibco Invitrogen Corporation, Carlsbad, CA, USA) with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L with the addition of 0.6 mL/L of gentamicin (Gibco Invitrogen Corporation, Carlsbad, CA, USA) and 10% fetal bovine serum (Gibco Invitrogen Corporation, Carlsbad, CA, USA).

MRC-5 (ATCC, Manassas, VA, USA); Human lung cells were grown in Eagle's Minimum Essential Medium (Gibco Invitrogen Corporation, Carlsbad, CA, USA) with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L with the addition of 0.6 mL/L of gentamicin (Gibco Invitrogen Corporation, Carlsbad, CA, USA) and 10% fetal bovine serum (Gibco Invitrogen Corporation, Carlsbad, CA, USA).

Mouse Hepatitis Virus strain MHV-1 (Parkes), Human Coronavirus OC43 (HCoV-OC43) and Human Coronavirus 229E (HCoV-229E) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

Cell Culture

Cell lines were grown in an incubator at 35.0°C ± 2.5°C supplemented with 5% CO_2. The cell lines were passaged by aseptically pipetting out all of the media and the cell surface was washed once with 1x PBS (Gibco Invitrogen Corporation, Carlsbad, CA, USA). Approximately, 1mL of trypsin-EDTA (1X) liquid (Gibco Invitrogen Corporation, Carlsbad, CA, USA) was added to the surface of the flask, spread evenly across the surface and removed. Another 1mL of trypsin-EDTA (1X) liquid (Gibco Invitrogen Corporation, Carlsbad, CA, USA) was added to flask and gently rocked back and forth to ensure the entire monolayer was covered. The flask was placed into the 35°C incubator until the cells detached. The activity of the trypsin was neutralized with the addition of the appropriate media for that cell line and 20% serum (v/v). The cell suspension was added to T75 flasks and placed back into the incubator to allow for cell growth. For the preparation of assay plates the cells were passaged as above but not placed into a T75 flask after detachment. The cell suspension was placed into a sterile 500mL flask with the

appropriate amount of volume of media plus 10% serum for 24 multi-well assay plates. 2mL of this suspension was added to each well of the 24 multi-well assay plates.

Virus Stocks

Cell monolayers demonstrating >90% confluency were used to produce virus stocks. All of the media was aseptically pipetted out of a T75 flask and 1 to 2 ml of the thawed virus was added to the confluent cells. The flask was rocked back and forth to ensure a complete coverage of the virus on the cell monolayer. The flask was placed in the 35˚C incubator for one to two hours for adsorption. After the adsorption period, 10 mL of media with 2% (v/v) serum was added into the flask and placed back into the incubator until $\geq$ 90% of the cell monolayer demonstrated the specific viral cytopathogenic effect (viral CPE). This usually took 24 to 48 hours. After this period the cell monolayer was removed from the plate using a cell scraper and the viral/cell suspension was collected and centrifuged at 2000 RPM (CRU-5000 Centrifuge) for 10 minutes at 4˚C. The supernatant was collected and serum was added so that the total volume of serum would be 10% (v/v). 1mL of this virus stock was added to cryogenic vials (Nalgene, Rochester, NY, USA) and stored at -75˚C.

EPA Virucidal Test Method

Multiwell plates (i.e. 24 well assay plates) containing the appropriate host cell line were prepared at least one day prior to the start of the assay procedure. On the day of the virucidal assay, growth media was aseptically removed from each well containing target cells and replaced with 2.0 mL of media containing 2% (v/v) horse serum. Media containing 2% (v/v) of serum (maintenance media) was used throughout the assay. The underside of a petri plate (100 x 15mm) was marked with an area of 28cm^2. An amount

of 0.2 to 0.4 mL of virus stock was spread over this area using a 10µl inoculating loop and allowed to dry for 30 to 50 minutes at room temperature until a white, dry film was apparent.

Test substance samples and sample dilutions were prepared using distilled (DI) water. For each sample tested, the test substance was applied directly to the virus film as per the label directions, and allowed to sit undisturbed for the 30 second contact time at an ambient temperature. After 5 seconds the virus/test substance mixture was resuspended by scraping the plate with a sterile cell scraper. Serial ten-fold dilutions were carried out in maintenance media (i.e. 0.2 mL of a virus dilution was added to 1.8 mL of maintenance media). A total of 0.2 mL of each dilution was then aseptically pipetted into each of four wells of host cells. The assay plates were incubated at 35˚C $\pm$ 2.5˚C supplemented with 5% CO_2 and the cells were observed for toxicity or characteristic viral cytopathogenic effect (CPE) by looking at the plates through a reverse microscope throughout the assay. The viral CPE was characteristic of a degenerated cell sheet and cell detachment. Maintenance media was changed after the first night of incubation by aseptically removing all of the media and replacing it with fresh 2 mL of maintenance medium. Additional refeedings were performed every 48-96 hours thereafter, as necessary. This was done by aseptically removing 1 mL of medium from each well, which had not demonstrated obvious cytotoxicity or viral CPE, and pipetting in a fresh 1 mL of sterile maintenance medium. The incubation time ranged between seven to ten days. Infectious dose titers were determined by the method of Reed and Muench ID_{50} endpoint method (42). The endpoint method is comprised of a statistical model to determine titers of viruses that could not be calculated by conventional plaque or focus

assays. Four replicates were performed for each dilution. In order to calculate the endpoint the cumulative number of infected and uninfected were reported in percentages. These numbers were used to calculate the proportionate distance factor which in turn was used along with the dilution factors to figure out the ID_{50} endpoint (i.e. the dilution that resulted in an infectious dose in 50% of the inoculated replicates). The endpoint of the test replicates were averaged between two replicates and then subtracted from the viral titer log recovery to determine the log reduction of each test substance per test.

In order to assess the viral titer, cells were assessed for viral infectivity in the presence of virus but in the absence of the test product. The above procedure was repeated except utilizing 2.0 mL of virus control resuspending medium (i.e. serum-free cell culture medium) in place of the test substance treatment. This control was used to verify the amount of virus present on the petri plate before disinfectant treatment. This endpoint became the log recovery and was used for all log reduction calculations.

In order to determine the effect of the test substance with the host, cells were assessed for toxicity by the product in the absence of virus. The above procedure was repeated utilizing 0.2 to 0.4 mL of toxicity control inoculum (i.e. cell culture medium + 10% serum) as the initial inoculum, in place of the virus stock. This control determines the effect of the disinfectant dilutions on the host cells in the absence of virus.

Host cells were assessed for general health in the absence of virus and product throughout the course of the assay period by ensuring the cell sheet did not detach and degeneration was not apparent. One row of cells (i.e. 4 wells) was left untreated (i.e. no inoculation).

EPA Virucidal Test Method using Sephadex Columns

Preparation of Sephadex Columns:

At least one day prior to testing, the sephadex slurry and columns were prepared. The sephadex slurry was prepared by adding 40 to 50g of lipophilic sephadex (Sigma-Aldrich, St. Louis, MO, USA) to 1000 mL of PBS solution. The solution was swirled to mix and remained in the refrigerator at least overnight. The slurry was then autoclaved for 20-25 minutes at 121°C/15 PSI to sterilize, and allowed to cool to ambient temperature prior to use. The columns were prepared by first inserting a small wad of glass wool into a 10mL syringe barrel and pushing it down to cover the opening in the syringe tip. The syringe barrel was then placed into a sterile 125 mL glass Gibco bottle, capped and autoclaved.

Sephadex columns plus the addition of the sephadex slurry were prepared on the day of the virucidal assay procedure. Sephadex slurry was pipetted into the barrel and permitted to drain, allowing the sephadex to settle. Sephadex slurry was added until the column height reached 9-11mL in a 10mL syringe barrel. Once the appropriate column height was reached, the addition of 10mL of cell culture medium was added to equilibrate the column. Prior to the start of the virucidal assay procedure, the filled columns were centrifuged at 700-900 RPM in a CRU-5000 centrifuge with a swinging bucket rotor for approximately 3-4 minutes to eliminate the void volume in the column.

During the neutralization step of the test substance / virus suspension or for the controls the 2mL's were added to a sephadex column and centrifuged for 3 to 3.5 minutes at 700 to 900 RPM in a CRU-5000 centrifuge. The column flow-through was collected and this was used for inoculation into the 10^{-2} wells and also serial ten-fold dilutions were

carried out in maintenance media (i.e. 0.2mL of a virus/test substance dilution was added to 1.8mL of maintenance media). A total of 0.2 mL of each dilution was aseptically pipetted into each of four wells of host cells. The assay plates were incubated at 35°C ± 2.5°C plus 5% CO2 and the cells were observed for toxicity or characteristic viral CPE throughout the assay. Refeedings were performed the same as above.

RNA Purification

After infectivity tests were completed these samples were collected for RT-PCR assays. Samples to be tested were thawed and 0.5ml of sample was added to a 1.5mL epindorf tube, using RNase free materials as necessary throughout, with 0.5ml of trizol (Gibco BRL, Gaithersburg, Maryland, USA) was added. Samples were mixed by pipeting up and down repeatedly and then incubated at room temperature for 5 minutes. After incubation, 0.1ml of chloroform (J.T. Baker Inc., Phillipsburg, NJ, USA) was added, shaken vigorously for 10 seconds and incubated at room temperature for 2 minutes. At 4°C, the sample was centrifuged for 15 minutes at 12000 rpm. After centrifugation the aqueous top phase was transferred to another 1.5 ml epindorf tube and 0.25 ml of isopropyl alcohol (Curtin Matheson Scientific, Houston, TX, USA) was added and incubated at room temperature for 15 minutes. Samples were centrifuged at 4°C for 10 minutes at 12000 rpm. The supernatant was removed and 1ml of 75% ethanol (Carolina Biological Supply Company, Burlington, NC, USA) was added, vortexed, and spun for 5 minutes at 7500 rpm at 4°C. The supernatant was removed and spun at 12000 rpm for 5 minutes. The supernatant was removed again and 50µl of RNase free water (Promega, Madison, WI, USA) was added to the tube.

RT-PCR Reaction

The Brilliant® SYBR® Green QRT-PCR Master Mix Kit, one step reaction was used and a RNase free procedure was followed throughout. A master mix was prepared for each 10 reactions using 74.4λ of RNase free water (Promega, Madison, WI, USA), 0.625 λ of StrataScript RT/RNase block enzyme mixture (Strategene, San Diego, CA, USA) and 125λ of 2x SYBR QRT-PCR master mix (Strategene, San Diego, CA, USA). Reactions were set-up to contain varying amounts of purified RNA and different primer sets. The three primer sets are used are described below.

1) IN-2: GGG TTG GGA CTA TCC TAA GTG TGA (6.66 nmole)

IN-4: TAA CAC ACA AAC ACC ATC ATC ATC A (9.3 nmole)

2) IN-6: GGT TGG GAC TAT CCT AAG TGT GA (6.91 nmole)

IN-7: CCA TCA TCA GAT AGA ATC ATC ATA (8.51 nmole)

3) BNIoutS2: ATG AAT TAC CAA GTC AAT GGT TAC (6.02 nmole)

BNIoutAS: CAT AAC CAG TCG GTA CAG CTA (8.09 nmole)

All primers were diluted 1:100 with the addition of RNase free water.

RT-PCR reactions were set-up for a total of 25λ in each reaction tube. Reaction tubes either contained 0λ, 1λ or 3λ of purified RNA with 1λ of each primer from the primer set, 20 λ of master mix and if necessary RNase free water to total the reaction to 25λ.

RT-PCR cycling program as follows: 1 cycle for 30 minutes at 45°C, 1 cycle for 10 minutes at 95°C and 40 cycles with 30 seconds of denaturing at 95°C, 1 minute of annealing at 60°C and 30 seconds of extension at 72°C.

Results

In order to determine the effectiveness of common household disinfectants and antiseptics on a SARS-like Coronavirus, MHV was analyzed for its ability to infect cells after treatment of household products used from North America, Europe and Asia. Human Coronavirus 229E, from the antigenic Group I of the *Coronaviridae*, along with its host, MRC-5 (Human lung cells) was used because this virus along with another type of Human Coronavirus causes 30% of colds every year in humans. Initially, it was planned to start with a virus that is known to cause infections in humans and then look at an animal virus. During viral propagation it became apparent that the cytopathogenic effect of Human Coronavirus 229E was not visible. Viral infection was performed three to four times after cell passaging and still viral CPE was not present. It has been previously determined that Human Coronavirus 229E has an established viral infection that allows for the virus to replicate without a noticeable viral CPE for an undetermined about of passages and therefore was not used throughout this study (4). Another coronavirus, HCoV-OC43, did not produce a visible CPE in cell culture using BSC-1 cells because the host chosen was not adapted to tissue culture methods and therefore could not be used. Further tests are necessary using other cell lines that will produce a viral CPE in order to study the antiviral action of common household products against the aforementioned types of Human Coronaviruses.

Murine Hepatitis Virus and its Cytopathogenic Effect

The presence of Murine Hepatitis Virus (MHV) infection was detected by observing viral cytopathogenic effect (CPE) of the NCTC clone 1469 cell line through an inverted microscope. CPE can occur because of toxicity of the product to the cells or

because of the presence of viral particles. The difference between viral CPE and cytotoxicity CPE can be determined by the controls that are performed during the test. Figure 1 demonstrates the cell line 8 hours after being passaged and only 50% confluent. The cells are healthy with rounding and a distinct morphology. Figure 2 – shows the cell line after 36 hours of being passaged and 80% confluent. Figure 3 indicates viral CPE and the effect the MHV has on the NCTC clone 1469 cell line. The cells no longer have that distinct form and structure causing degeneration of the cell sheet.

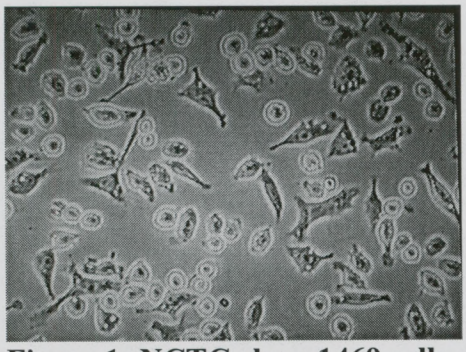


Figure 1*:* NCTC clone 1469 cells after 8 hours of cell passaging

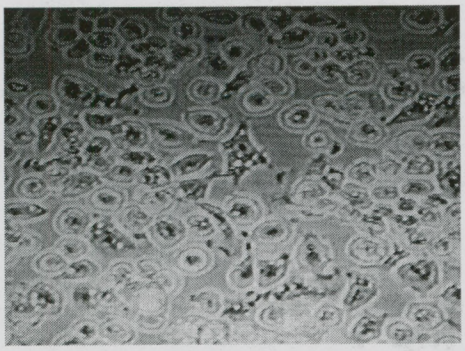


Figure 2*:* NCTC clone 1469 cells after 36 hours of cell passaging

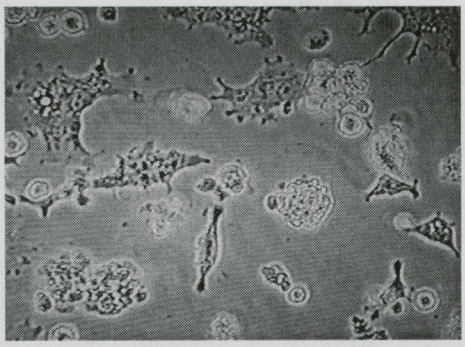


Figure 3: NCTC clone 1469 cells infected with MHV

Disinfectant and Antiseptic Products

Since successful viral propagation occurred with MHV this virus was used to measure the antiviral properties of various household disinfectants and antiseptics. A list of the products tested, the dilutions used during testing and the yielding concentrations is outlined in Table 1. The products chosen were based on their use in specific parts of the world, especially those affected by the 2003 SARS outbreak, and also by the varying active ingredients to determine the virucidal activity. It is important to note that most of the products tested were dilutables and require a specific dilution to be effective. The dilutions were chosen based on manufacturer's instructions, except for the liquid hand soap. The liquid hand soap dilution was chosen to allow for the thick product to be dilute enough to assess its efficacy but not too dilute that invalid test results would be obtained. A 30 seconds contact time was used because this was the quickest time one operator can perform while carrying out the EPA virucidal test method.

Table 1 - Product Summary with the Concentration of the Active Ingredient

Product	Active Ingredient	Dilution	Final Concentration Tested	Location of Product
Lysol Disinfectant Spray®	0.1% alkyl dimethyl benzyl ammonium saccharinate / 79% ethanol	N/A	1000ppm / 790,000ppm	North America
Dettol Brown Liquid Antiseptic / Disinfectant®	4.8% chloroxylenol (PCMX)	1:40	1200 ppm	Europe and Asia
Household bleach	5.25% sodium hypochlorite	1:25	2100 ppm	All
Clean & Smooth Soap®	0.2% triclosan	1:4	500 ppm	North America
Pine-Sol®	15.0% pine oil	1:64	2343 ppm	North America

EPA Virucidal Test Method Requirements and Test Results

Evaluation of the effectiveness of the products being tested and the validity of the assay were accepted when the following criteria were met:

1. A minimum viral titer of 10^4 was recovered from the test surface (i.e. virus control).
2. Inactivation of the virus by the test substance at all dilutions as measured by the viral CPE not being present (i.e. the cells with the viral/test substance suspension).
3. When cytotoxicity was observed in the test system, at least a 3 log reduction in the viral titer had to be demonstrated.
4. The host cells remained healthy and viable throughout the course of the assay period as determined by the control wells which did not have any viral suspension or product added to the cells.

An EPA test method only requires a product to be tested once with two replicates in order to be proven affective. However, in order to achieve an 80%

confidence interval, it was determined that the products should be tested five times with two replicates for each test (Table 2).

Table 2 – Raw Data: Reduction in Virus infectivity after a 30 second contact time; Calculations were done using the Reed and Muench ID50 endpoint (42)

Product	MHV Recovery	Virus Present: rep.1 and rep. 2	CPE due to toxicity of product	Log Reduction
Lysol Disinfectant Spray® (alkyl dimethyl benzyl ammonium saccharinate / ethanol)	Test 1*: 5.50	no	≤2.50	≥3.00
	Test 2*: 5.50	no	≤2.50	≥3.00
	Test 3*: 5.50	no	≤2.50	≥3.00
	Test 4*: 5.50	no	≤2.50	≥3.00
	Test 5*: 5.50	no	≤2.50	≥3.00
Dettol Brown Liquid Antiseptic/ Disinfectant® (PCMX)	Test 1: 5.67	no	≤1.50	≥4.17
	Test 2: 6.00	no	≤1.50	≥4.50
	Test 3: 6.00	no	≤1.50	≥4.50
	Test 4: 6.00	no	≤1.50	≥4.50
	Test 5: 6.00	no	≤1.50	≥4.50
Household Bleach (sodium hypochlorite)	Test 1: 5.50	no	≤1.50	≥4.00
	Test 2: 6.00	no	≤1.50	≥4.50
	Test 3: 6.00	no	≤1.50	≥4.50
	Test 4: 6.00	no	≤1.50	≥4.50
	Test 5: 6.00	no	≤1.50	≥4.50
Clean & Smooth® (triclosan)	Test 1*: 6.33	no	≤1.50	≥4.83
	Test 2*: 6.33	no	≤1.59	≥4.74
	Test 3*: 6.33	no	≤2.00	≥4.33
	Test 4*: 6.33	no	≤1.50	≥4.83
	Test 5: 5.50	no	≤2.50 (no sephadex)	≥3.00
Pine-Sol® (pine oil)	Test 1: 5.67	no	≤1.50	≥4.17
	Test 2: 6.00	no	≤1.50	≥4.50
	Test 3: 6.00	no	≤1.50	≥4.50
	Test 4: 5.50	no	≤1.50	≥4.00
	Test 5: 5.50	no	≤1.50	≥4.00

*Test procedure performed using sephadex columns.

The CPE due to the toxicity effect of the product was determined 24 hours after the test and was based on two controls. The virus controls performed had varying dilutions of the virus and plated into the multi-well assay plates containing the preferred

cell line. This control did not contain any product and determined the CPE due to the presence of the virus. The toxicity controls had the product at varying dilutions, no virus present, and plated into the assay plates. This determined the CPE due to the presence of the toxicity from the product.

When a 3-log reduction of the virus could not be achieved, sephadex, as noted in Table 2, was performed with Lysol Disinfectant Spray® and Clean and Smooth Soap®. This occurred due to the product cytotoxicity and not because of the effectiveness of the product. The sephadex column has a cross-linked dextran bases resin that produces an affinity for lipids and reduces the amount of active that has a cytotoxicity affect on the cell line. Sephadex columns are used after neutralization has occurred and are even used for the virus and toxicity controls. Therefore, the product is being assayed for it's efficacy at this point and there will not be any considerations to the further diluting of the active.

Calculations were performed using the Reed and Muench ID_{50} endpoint method. This calculation method can only be performed on viruses that cause CPE in cultured cells and an adequate amount of incubation time is given to allow for infection to take place. The samples being tested for infectivity were diluted and inoculated into the NCTC clone 1469 cell line. Four replicates were performed for each dilution. In order to calculate the endpoint the cumulative number of infected and uninfected were reported in percentages. These numbers were used to calculate the proportionate distance factor which in turn was used along with the dilution factors to figure out the endpoint.

At least a 3-log reduction along with the inactivation of the virus from all dilutions are two of the requirements that are important at determining a product effective against a virus and necessary to attain EPA registration status.

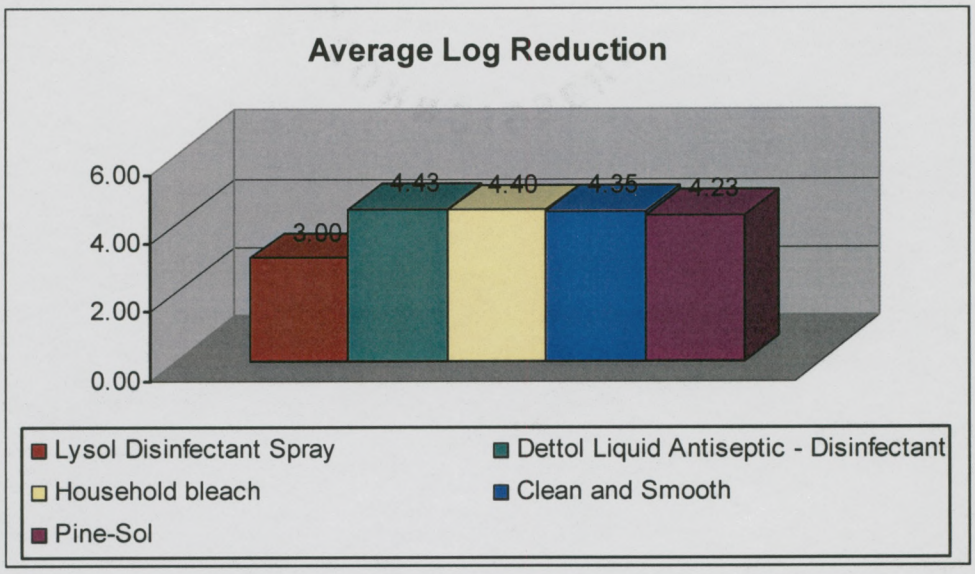
A summary of the averages of the five tests for each product has been outlined in table 3. This table demonstrates that in fact all of the common household disinfectants / antiseptics were effective at eliminating viral particles from a hard non-porous surface by providing a 3-log reduction or greater of the virus from the surface.

Table 3 – Averages of the Virus Recovery, the amount of wells present with viral particles, average log reduction and the final result

Product	MHV Recovery	Virus Present in any wells	Average Log Reduction	Result
Lysol Disinfectant Spray® (alkyl dimethyl benzyl ammonium saccharinate / ethanol)	5.50	No	≥3.00	Effective
Dettol Brown Liquid Antiseptic / Disinfectant® (PCMX)	5.93	No	≥4.43	Effective
Household Bleach (sodium hypochlorite)	5.90	No	≥4.40	Effective
Clean and Smooth® (triclosan)	6.16	No	≥4.35	Effective
Pine-Sol® (pine oil)	5.73	No	≥4.23	Effective

The log reductions obtained from the certain active and its concentration along with the amount of log reduction achieved is illustrated in figure 4. The lower the log reduction does not mean that the product was less effective because some of the dilutions were eliminated due to cellular toxicity of the product and not viral CPE.

Figure 4 - A comparison of the log reductions of the common household disinfectant / antiseptic products used against MHV



The research showed that Lysol Disinfectant Spray® (0.1% alkyl dimethyl benzyl ammonium saccharinate and 79% ethanol), Dettol Liquid Antiseptic – Disinfectant® (chloroxylenol B.P. 4.8%), household bleach (5.25% sodium hypochlorite), Clean & Smooth Soap® (0.2% triclosan) and Pine-Sol® (pine oil 15%) were effective against MHV. The reduction in virus titer for the test substance was greater than or equal to 3 logs of inactivation and the virus titer recovered from the carrier exceeded 10^4. All products demonstrated complete inactivation of the MHV at all dilutions assayed. This research has proven that when used properly, common household disinfectants will eliminate Murine Hepatitis Virus particles from the surface.

Table 4: Products tested outside of the recommended dilution

Product	Dilution	Virus Present in any wells	Result
Lysol Disinfectant Spray® (alkyl dimethyl benzyl ammonium saccharinate / ethanol)	1:10	Yes	Not Effective
Dettol Brown Liquid Antiseptic / Disinfectant® (PCMX)	1:1000	No	Effective
Household Bleach (sodium hypochlorite)	1:250	Yes	Not Effective
Clean and Smooth® (triclosan)	1:25	Yes	Not Effective
Pine-Sol® (pine oil)	1:1000	No	Effective

The disinfectants and antiseptics were also tested outside of the recommended dilution to assess how effective the products would be against MHV if they were not used correctly. This information is useful to know for infection control procedures because this indicates how far the product can be diluted before loss of efficacy. As one can see Dettol Liquid® and Pine-Sol® contain actives that remain active even at very high dilutions. Household bleach being one of the most common products used for infection control becomes ineffective as a virucidal agent against MHV if not diluted properly. Lysol Disinfectant Spray® and Clean and Smooth® were also not effective at eliminating MHV from the surface when used incorrectly. Lysol Disinfectant Spray® is not a dilutable product but in order to obtain data a sample was collected in a tube by spraying for 10 to 15 seconds and then it was further diluted.

RT-PCR Results

Table 5: RT-PCR data

Primer Set	Ct of 0µl RNA	Ct of 1µl RNA	Ct of 3µl RNA
1: IN-2 and IN-4	26.26	24.14	24.55
2: IN-6 and IN-7	30.95	26.59	25.11
3: BNIoutS2 and BNIoutAS	28.09	27.31	27.98
1 (rep. 2)	25.83	25.35	17.51
2 (rep.2)	29.98	23.87	22.02
3 (rep.2)	27.91	28.06	28.38

The RT-PCR data is represented by the amount of cycles necessary for the fluorescence to be detected passed a set threshold. Three sets of primers were used to detect which primer set would be the best at replicating the MHV RNA used in these experiments. The MHV controls were used a varying amounts (i.e. 0, 1 and 3 µl) and the one step SYBR reaction was used. The more amount of RNA that is present the less time or cycles it would take for that sample to fluoresce passed the threshold. Therefore one would expect the Ct values to decrease when more MHV RNA is present. A greater than five Ct value between the no template control and the varying amounts of templates is used to deem valid results and represents a 32 fold difference. This difference is consistently only seen in primer set #2 for both replicates from the 0µl to the 1µl of MHV RNA and would be considered the best option of primer sets to use throughout the other RT-PCR reactions.

Conclusion

The 2003 outbreak of Severe Acute Respiratory Syndrome (SARS) infected over 8,000 people and killed 784 leaving many questions to how effective are household disinfectants and antiseptics at eliminating viral particles from surfaces and preventing transmission. This research proved that Lysol Disinfectant Spray®, Pine-Sol®, Clean & Smooth Antibacterial Soap®, Dettol Liquid ® (a brand well-known and frequently used in parts of the world where the SARS outbreak occurred) and household bleach were effective against Murine Hepatitis Virus (MHV), a virus that is closely related to SARS.

In an outbreak caused by novel agents, such as SARS, it is important to know the effectiveness of disinfectants/antiseptics to prevent or reduce the possibility of human- to-human transmission by means of surfaces. However, there are many safety precautions that have to be followed when testing the SARS virus, making it difficult to prove the efficacy of these preventative reagents.

Because many viruses are dangerous to work with, a surrogate virus is often identified to study with. Genetic analysis performed on SARS identified the virus as a member of the Coronavirus family. Coronaviruses in general are more commonly associated with causing respiratory and enteric diseases and in humans causes 30 % of mild upper respiratory tract infections (4). MHV was chosen as a surrogate virus because it demonstrates a close relation to SARS from the Coronavirus family and also, because of the accomplished viral CPE in cell culture. This allowed for proficiency during testing and enabled consistent results. MHV would serve as a predictor of how household disinfectants and antiseptics would perform against SARS.

This data shows that common household disinfectants and antiseptics are effective at eliminating MHV from surfaces using a method that closely follows the guidelines established by the federal government for a standard virucidal assay. All household products tested demonstrated a $\geq$99.9% inactivation of MHV. Therefore a connection can be made that if household cleaners are effective at eliminating MHV from surfaces then they should also be effective at inactivating the SARS virus. This demonstrates the ability that disinfectants and antiseptics have at preventing transmission of viruses via surfaces when used correctly. Also, a study performed with free chlorine and chlorine dioxide demonstrated its abilities at inactivating the SARS virus. The concentrations that succeeded at inactivating the SARS virus were not the same for gram negative organisms such as E. coli and proved that SARS is more susceptible to disinfectants then E. coli (21). It also states the importance of diluting the product correctly and letting it sit the correct about of time to allow for inactivation of the virus. Chlorine used at 10 mg/L with a contact time of 30 minutes produced a 100% inactivation of SARS but if that contact time was only 1 minute then only a 43.77% inactivation could be demonstrated (21).

Two of the products in this report were sent to a lab overseas to confirm this data but testing with the SARS-Urbani strain was used instead of MHV. The method aforementioned was used as a guideline as the labs were testing these products. Lysol Disinfectant Spray® and Dettol Liquid – Antiseptic and Disinfectant® were tested against the Urbani SARS virus at a 30 seconds and a 5 minute contact time. The same techniques were used against the SARS virus and as predicted the same results (i.e. a 99.9% reduction) were achieved by both products.

During the outbreak many of the other surrogate viruses (e.g. Human Coronavirus 229E and Human Coronavirus OC43) were backordered for months and when testing begun there were some difficulties with achieving CPE in cell culture. Therefore this allowed initiation of the infectivity tests to be performed against MHV. Samples from each test were stored frozen at -75°C to be used for RT-PCR analysis. This analysis will be useful in determining the mechanism of action, determining if RNA is still present and if present why it did not produce positive infectivity results in cell culture. The RT-PCR data thus far has helped to determine a primer set to be used to perform subsequent analysis on the frozen samples from each of the tests.

In less than four months, from the point of when a global alert was issued in March of 2003 to July 5th of 2003 it was declared that SARS was successfully contained. This is an attributable mark for which the scientific community handled an outbreak from a novel agent, not knowing the causative agent, not having any diagnostic tests or available treatments and even without proven effective infection control procedures. One can see the importance of an international collaboration during an outbreak. The SARS outbreaks lead to clinical, epidemiology and laboratory research worldwide. The global collaboration eventually led to the termination of SARS transmission. Other efforts that are still taking place are the determination of the origin of the SARS virus, its natural host, development of a vaccine or other antiviral compounds to treat the SARS virus and diagnostic tests to assist with rapid and reliable identification. This research has proven the advantages of a surrogate virus during an outbreak of a new and emerging virus. The information learned from the surrogate can be used to help with the understandings of the current unknowns of the new virus.

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