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Major article

Efficacy of common disinfectant/cleaning agents in inactivating murine norovirus and feline calicivirus as surrogate viruses for human norovirus

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Background: The efficacies of disinfection by sodium hypochlorite, accelerated hydrogen peroxide (AHP), and quaternary ammonium compound (QUAT) commonly used in health care facilities were determined using the surrogate viruses murine norovirus (MNV-1) and feline calicivirus (FCV).

Methods: A virus suspension of known concentration (with or without a soil load) was deposited onto stainless steel discs under wet or dry load conditions and exposed to defined concentrations of the disinfectant/cleaning agent for 1-, 5-, or 10-minute contact time using the quantitative carrier test (QCT-2) method. Virus inactivation was determined by plaque assay.

Results: At an exposure time of 1 minute, sodium hypochlorite at 2,700 ppm was able to inactivate MNV-1 and FCV with a >5 $\log_{10}$ reduction. After 10 minutes, MNV-1 was inactivated by AHP at 35,000 ppm, whereas FCV was inactivated at 3,500 ppm. MNV-1 was not inactivated by QUAT at 2,800 ppm. A QUAT-alcohol formulation containing 2,000 ppm QUAT and 70% ethanol was effective in inactivating MNV-1 after 5 minutes, but resulted in only a <3 $\log_{10}$ reduction of FCV after 10 minutes.

Conclusions: AHP and QUAT products were less effective than sodium hypochlorite for the inactivation of MNV-1 and FCV.

Noroviruses are single-stranded, positive-sense RNA viruses that belong to the *Caliciviridae* family.[1] It is estimated that each year, more than 21 million cases of acute gastroenteritis are caused by norovirus infection.[2]

Human norovirus is highly infectious, producing such symptoms as nausea, abdominal cramps, vomiting, and watery diarrhea.[3] Norovirus has a low infectious dose, and thus individuals ingesting as little as 10-100 virus particles may become infected.[3,4] It has been known to survive on human hands, environmental surfaces, and inanimate objects, where it may remain infectious for up to 28 days.[5,6] With a low infectious dose and fecal–oral transmission route, contamination of environmental surfaces plays an important role in the generation of norovirus outbreaks.[6]

Because human norovirus cannot be cultivated in cell culture, detection and quantitation of this agent is accomplished by real-time reverse-transcription polymerase chain reaction (RT-PCR) assays. Compared with, for example, the plaque assay, RT-PCR is not feasible for distinguishing between infectious and noninfectious virus, however.[7,8] Thus, cultivable surrogate viruses, namely the murine norovirus (MNV-1) and feline calicivirus (FCV), have been used to study the persistence, inactivation, and transmission of human norovirus.[9,10] Although these surrogates are classified into the same family, they differ in their response to pH, organic solvents, temperature, and environmental surface conditions, and might not accurately reflect the environmental survival and susceptibility to disinfection of human norovirus.[9-11] MNV-1 can survive at ambient temperatures and in a spectrum of pH

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environments, from pH 2 to 10, whereas FCV is more susceptible to pH extremes (pH 2, 3, and 10) and is less stable under dry conditions compared with MNV-1.[9-12]

The purpose of the present study was to determine the efficacy of disinfectants under conditions and methodology commonly used in health care facilities in British Columbia, Canada. Commonly used disinfectants include quaternary ammonium compound (QUAT) products, sodium hypochlorite (bleach), and accelerated hydrogen peroxide (AHP). Previous studies have evaluated the effectiveness of different types of disinfectants using either MNV-1 or FCV, but not both together. Coupled with different methodologies, environmental conditions, and contact times, it is difficult to compare survival of these surrogate viruses against the efficacy of disinfectants. This study was taken a step further to include 2 environmental conditions, viral survival with and without a soil load. Three contact times were selected to determine the extent to which viral survival was affected by duration of exposure to the disinfectants.

MATERIALS AND METHODS

Viruses and cell cultures

MNV-1, FCV strain F9 (ATCC VR-782), macrophage mouse RAW 264.7 cell line (ATCC TIB-71), and Crandell Rees feline kidney (CRFK) cell line (ATCC CCL-94) were purchased from the American Type Culture Collection (ATCC; Manassas, VA).

Disinfectants and cleaning agents

Five disinfectant/cleaning agents were used in this study. All products were tested within the manufacturer's expiration date and stored at room temperature. Dilutions from concentrated disinfectants were prepared on the day of the experiment and used within 2 hours. Disinfectant solutions were diluted at room temperature using distilled, deionized water, and mixed for 1 minute before use. Ready-to-use (RTU) disinfectants were used undiluted.

The following products were selected for this study:

- Domestic Miraclean Bleach (ClearTech Industries, Vancouver, BC, Canada) containing 5.4% chlorine
- Accel (Virox Technologies, Oakville, ON, Canada; DIN 02245061) containing 7.0% AHP
- Virox 5 (Virox Technologies; DIN 02239775) containing 0.5% AHP
- CaviCide (Metrex Research, Orange, CA; DIN 02161656) containing 17.2% isopropanol and 0.28% QUAT (diisobutylphenoxyethoxyethyl dimethyl benzyl)
- $T^3$6 (ALDA Pharmaceuticals, New Westminster, BC, Canada; DIN 02231344) containing 70% ethanol, 0.28% *o*-phenylphenol, 0.01% chlorhexidine gluconate and 0.20% QUAT (benzalkonium chloride)

Virus inactivation tests

The stainless steel disc-based quantitative carrier test 2 (QCT-2) method was followed with modifications, such as the addition of wet load conditions and the absence of a soil load.[13] MNV-1 and FCV were tested in triplicate under wet and dry load conditions, with and without a soil load. For this, 10 μL of virus was added onto autoclaved 1-cm-diameter stainless steel discs, followed by exposure to wet or dry load conditions. To achieve dry load conditions, virus inoculum was dried for 60-90 minutes in a biosafety laboratory cabinet at room temperature; for wet load conditions, this drying step was omitted. To each stainless steel disc carrier containing the virus, 50 μL of test biocide was added. The discs were held for 1, 5 or 10 minutes at room temperature, after which the disinfectant was inactivated by the addition of 950 μL of neutralizer. The volume of QUAT products containing alcohol was reduced to 20 μL per 980 μL of neutralizer. Earle's Balanced Salt Solution (Gibco 14155-063; Life Technologies, Carlsbad, CA) served as the control for the biocide. The viral titer was determined by plaque assay. Cytotoxicity of the test formulation, neutralizer, and the combination of both mixtures was evaluated as described by Sattar et al.[13]

Soil load mixture

Preparation of a 500-μL virus inoculum containing soil load involved 100 μL of bovine mucin (0.004 g/mL), 35 μL of tryptone (0.05 g/mL), and 25 μL of bovine serum albumin (0.05 g/mL) added to a 340 μL of virus suspension. All soil load components were prepared in phosphate-buffered saline.[13]

Neutralizer

Neutralizer was used to arrest the virucidal activity at the end of the defined contact time. Sodium thiosulphate pentahydrate (BDH Chemicals, Toronto, ON, Canada) at 2.5% was used as a neutralizer for sodium hypochlorite[14]; 10.3% Letheen broth (95039-384; HiMedia Laboratories, Mumbai, India) with 2.0% sodium thiosulfate was used as a neutralizer for QUATs and AHP.[15] Neutralization was accomplished through a combination of chemical neutralization and serial dilutions.

Cytotoxicity control tests

Tests were performed to determine which disinfectant formulation concentrations produced no cytotoxicity in the cell line and to assess whether the neutralizer reduced or enhanced the cytotoxicity of the cell line.[13]

Plaque assay

The plaque assay method used in this study was based on the protocols of Bidawid et al.[8] and Wobus et al.[16] with minor changes to the viral plaque incubation period. RAW 264.7 and CRFK cells were adjusted to a density of 2.5×10^6 viable cells in tissue culture 6-well plates (BD Falcon, 353846; BD Biosciences, San Jose, CA) and incubated at 37°C in a CO_2 incubator to ~90 % confluence. Serial 10-fold dilutions of the virus were prepared, and 500-μL aliquots of each dilution were added in triplicate to well plates and incubated at room temperature for 1 hour.

After incubation, aliquots were removed, and 47°C agarose containing equal volumes of 1.25% SeaPlaque agarose (50101; Lonza, Rockland, ME) and 2X minimum essential medium (Gibco12000-063; Life Technologies) with 10% fetal bovine serum (Gibco 12483-020; Life Technologies) was added to each well. Plates were incubated in a 37°C CO_2 incubator for 36-40 hours (MNV-1) or 40-45 hours (FCV), and overlaid with agarose containing 0.1% neutral red (57993-100ML-F; Sigma-Aldrich, St Louis, MO). Plates were reincubated for up to 24 hours, and plaques were counted. MNV-1 plaques were counted at 5-8 hours and 24 hours after the addition of overlay, and FCV plaques were counted at 24 hours. Test and control plaques were counted to determine the virus titer, which was expressed as plaque-forming units (PFU) per milliliter.