

Virucidal activity of a quaternary ammonium compound disinfectant against feline calicivirus: A surrogate for norovirus

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Background: Norovirus, formerly known as Norwalk virus, is an important cause of gastroenteritis outbreaks in hospitals, food services, schools, and cruise ships. Infection control practices by using disinfectants to eliminate noroviruses from surfaces and environmental samples reduce the morbidity and spread of virus outbreaks. There are not many commercial disinfectants effective against norovirus. Noroviruses cannot be cultivated in vitro. However, feline calicivirus can be used as a surrogate to determine disinfectant efficacy against noroviruses. Feline calicivirus was used in a virucidal effectiveness test protocol as a surrogate for norovirus to determine the virucidal efficacy of R-82, a quaternary ammonium compound disinfectant cleaner.

Methods: Feline calicivirus suspensions containing at least 5% fetal bovine serum were dried on carriers and treated with 1:256 dilutions of R-82 disinfectant in water, with a hardness of 400 ppm as calcium carbonate, for 10 minutes. Hypochlorite concentrations of 100 ± 10 and 1000 ± 10 ppm, respectively, were also analyzed as internal control standards. After contact period, the test agents were neutralized with 2 mL of appropriate neutralizer, and mixtures were scraped from carrier surfaces with a cell scraper. Selected dilutions of the neutralized inoculum/test agent mixtures were added to cultured cell monolayers of appropriate host cells. Postincubation, the infectious feline calicivirus was scored microscopically by observing virus-specific cytopathic effects produced by replicating infectious virus. The performance criterion was a minimum of 4-log10 reduction in cytopathic effects of feline calicivirus.

Results: After a 10-minute contact time, formulation R-82 diluted 1:256 showed a 6.6- and 6.4-log10 reductions in cytopathic effects of feline calicivirus during initial and confirmatory testing, respectively, demonstrating complete inactivation of the virus. A hypochlorite solution of 1000 ppm exhibited similar log10 reductions to the quaternary ammonium disinfectant, demonstrating the reproducibility of the protocol.

Conclusion: Formulation R-82, a quaternary ammonium compound, is a 1-step disinfectant cleaner, which exhibited virucidal activity against feline calicivirus suspensions dried on hard surface carriers. Surfaces are vectors for virus transmission during outbreaks by transferring the virus to people or other environmental surfaces. Therefore, treatment of contaminated surfaces with formulation R-82 will optimize disinfection of noncritical and critical surfaces in health care institutions, reducing the possibility of virus transmission during outbreaks. (Am J Infect Control 2006;34:269-73.)

Nonbacterial acute gastroenteritis outbreaks affect health care institutions, homes, food services, schools, and cruise ships. The number one microbial agent responsible for nonbacterial acute gastroenteritis is the norovirus (NV) formerly known as Norwalk virus. The NV is transmitted predominantly through the fecal-oral route, directly from person to person, through contaminated food or water, or by contact with contaminated surfaces or fomites.1-11 Aerosolized vomitus also has been implicated as a transmission mode.12,13 Because of high infectivity, e.g., low infectious dose, and persistence in the environment, it is important to implement proper infection control procedures to minimize the morbidity and transmission of noroviruses from surfaces and environmental samples. Several studies have demonstrated the transmission of the virus through inanimate surfaces.2-4 Disinfection procedures require the treatment of surfaces and environmental samples with efficacious disinfectants to inactivate and/or kill the virus.14-17 However, because NV cannot be grown in tissue culture, disinfection efficacy and inactivation studies have been performed using closely related viruses such as feline calicivirus (FCV) and canine calicivirus as surrogates for human NVs.14,15,18 It has been reported that quaternary ammonium compound (QAC)-based disinfectants are not efficacious against Noroviruses or FCV.15,19-21 The major objective of this study was to determine the virucidal activity of a QAC disinfectant cleaner, formulation R-82, against FCV, a surrogate of the norovirus.
METHODS

All virology testing and feline calicivirus protocol development was performed at Microbiotest, Inc., Sterling, VA.

Inoculum preparation

Virus stocks were propagated as previously described. The organic soil concentration in the virus stock was adjusted to a minimum of 5% organic soil (fetal bovine serum, Quad Five, Ryegate, MT).

Carrier preparation

An aliquot of 0.2 mL stock virus was spread over an area approximately 4 inches square that was marked on the underside of presterilized petri dishes with a cell scraper. The virus was allowed to dry for 30 to 60 minutes at room temperature. Two carriers were prepared for each lot of formulation R-82 (Lonza, Inc, Allendale, NJ), data consistency concentration, and plate recovery control. One carrier per test agent lot and each data consistency concentration were prepared for neutralizer effectiveness control using cell culture medium (CCM) in place of stock virus.

Test agent preparation

A 1:256 dilution of formulation R-82 in water with a hardness of 400 ppm as calcium carbonate (CaCO₃) was used. When diluted 1:256, the final QAC concentration in the test dilution was 850 ppm. Three different lots were tested. The lot numbers were TRCS No. 140023, TRCS 140024, and TRCS 140025.

Disinfection test protocol

The test protocol consisted in the simulation of in-use conditions: the FCV was inoculated onto hard surfaces (presterilized petri dishes), allowed to dry, and then treated with 2 mL of test product ensuring that the dried virus is completely covered. The plates remained at the temperature and contact time specified (20°C ± 2°C, 10 minutes). After contact period, the test agent was neutralized with 2 mL of appropriate neutralizer, and the mixture was scraped from the surface of the dish with a cell scraper.

Infection, cell maintenance, and infectivity assays

Selected dilutions of the neutralized inoculum/test agent mixture were added to cultured cell monolayers of Crandell-Reese feline kidney cells. Four wells per dilution were added to the host cell monolayers and incubated at 37°C ± 2°C in 5% ± 1% CO₂ for 2 to 3 days. Postincubation, the infectious FCV was scored microscopically by observing virus-specific cytopathic effects produced by replicating infectious virus.

Controls

Cell viability control demonstrates that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the CCM employed throughout the assay period. Plate recovery control (PRC) determines the relative loss in virus infectivity resulting from drying and neutralization alone. Column titer was performed to determine any effects of Sephadryl columns on infectious virus titer while passing through the columns.

In the neutralizer effectiveness control, each lot of the test agent was processed exactly as the test procedure but, instead of viral inoculum, CCM was added. Postneutralization, a 1-mL sample was divided into 2 portions (1 for cytotoxicity control [see below] and 1 for neutralizer effectiveness). Each sample was then passed through individual columns, and the eluate was serially diluted 10-fold in CCM. Following this procedure, virus (100 μL stock) was added to each dilution and incubated at the contact conditions outlined for a period equivalent to or greater than the contact time. Next, the selected dilutions were used to inoculate host cells as described for the test procedure.

Cytotoxicity controls were performed to determine whether the product was toxic to the cells. Each lot of the neutralized test agent and each concentration of the internal control test agent were run to determine cytotoxicity.

Data consistency control/internal control test agent (method validation and positive control) were performed when dried challenge virus were exposed to 2 mL/carrier of different concentrations of sodium hypochlorite solution in place of the test agent. After a contact time of 10 minutes at room temperature, each carrier was neutralized using 2 mL minimum essential Eagle’s medium with 10% fetal bovine serum (FBS) and 0.3% Na₂S₂O₃. These results obtained from this control provided data to demonstrate the ability of the protocol to generate reproducible, valid data.

The log reduction for each test and control culture was determined from the dilutions plated using the method as described in the Virucidal Testing Format and Statistics Primer issued by the Environmental Protection Agency (EPA) March 2000, based on statistical analyses previously described. According to the EPA, the test agent passes the test if a minimum of 4-log₁₀ reduction in cytopathic effects of FCV (complete inactivation of the virus occurs at all dilutions tested) is demonstrated compared with plate recovery control. When cytotoxicity is present, at least a 3-log₁₀ reduction from the PRC must be demonstrated beyond the
cytotoxic level with complete inactivation of the virus at all dilutions tested.

RESULTS

The results for the initial testing of formulation R-82 against FCV are shown in Table 1. Each value shown for the samples treated with R-82 disinfectant is an average of 3 different batches performed in duplicate. After 10-minute contact time, a 1:256 dilution of formulation R-82 was capable of inactivating FCV on hard surface carriers in the presence of 5% organic soil. No virus was recovered in any of the plated dilutions as demonstrated by the lack of cytopathic effect in different dilutions of the inoculated cultures (Table 1). The log_{10} most probable number (MPN)/mL average of virus titer was found to be 7.8 (Table 1). The plate recovery and column titer controls were performed in duplicates. The average log_{10} MPN/mL for the column titer control was 6.8. Results for the PRC indicated that the average log_{10} MPN/mL was 6.4 (Table 2). The log_{10} reduction for FCV (test vs PRC) was determined to be 6.4 (Table 2). Again, all data consistency and other controls were satisfactory. The average log_{10} reduction for sodium hypochlorite against FCV was 2.8 for a 100 ± 10 ppm solution and 6.4 for a 1000 ± 10 ppm solution (Table 2).

DISCUSSION

One of the practices to control norovirus outbreaks and reduce the incidence of morbidity and transmission is to clean and disinfect contaminated surfaces and materials. Infection control professionals must stop the virus outbreak by implementing thorough cleaning and disinfection procedures that will eliminate virus particles from surfaces and environmental samples. Surfaces can be efficient vectors for norovirus transmission. NV has been demonstrated to be transferred from contaminated surfaces by fingers to surfaces, taps, door handles, and telephone receivers. Hospital outbreaks of norovirus can result in the closing of wards to eliminate virus transmission and provide time for environmental decontamination. Optimal hospital decontamination is based on the use of disinfectants with proven virucidal activity against NV.

The protocol used in this study was based on spreading and drying FCV, as a surrogate for NV, on hard surface carriers, followed by treatment with a dilution of the QAC disinfectant for 10 minutes. Previous studies using a 1:256 dilution of product with a shorter contact time of 5 minutes did not show inactivation of the virus. In this study, initial and confirmatory testing of 3 batches of formulation R-82 demonstrated that, after a 10 minutes contact time, a 1:256 dilution of formulation

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Samples treated with R-82 disinfectant*</th>
<th>Samples treated with NaOCl† (100 ± 10 ppm)</th>
<th>Samples treated with NaOCl† (1000 ± 10 ppm)</th>
<th>Plate recovery control†</th>
<th>Column titer control†</th>
<th>Virus stock titer†</th>
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The plus and minus signs represent 4 duplicates.

MPN, most probable number; PNS, postneutralized sample; ND, not determined; NaOCl, sodium hypochlorite; +, feline calicivirus-infected cells detected; cytotoxic effects observed; −, feline calicivirus-infected cells not detected; no cytotoxic effects observed; 0, no cytotoxicity observed.

*Averages of 3 batches performed in duplicates.
†Averages of duplicates.

Table 1. Virucidal effectiveness results of samples and controls for initial testing
R-82 was capable of inactivating FCV in the presence of 5% organic soil. No virus was recovered after a 10-minute contact with product as demonstrated by the lack of cytopathic effect in the inoculated cultures. During initial and confirmatory testing, the log$_{10}$ reduction for FCV was calculated as the difference between the test versus PRC. These values were determined to be 6.6 and 6.4, respectively. Although, a 1:10 dilution of the samples was not plated, even if cytopathic effects were obtained in that particular dilution, the product will still be virucidal because, to claim virucidal activity against FCV, the formulation must show a minimum log$_{10}$ reduction of 4.0.

A minimum of 1000 ppm of sodium hypochlorite is recommended by the Centers for Disease Control and Prevention for the disinfection of norovirus-contaminated hard surfaces. Because of hypochlorite virucidal activity against FCV, it was run as a data consistency control (positive). Two different concentrations of sodium hypochlorite were analyzed during this study. The concentrations were 100 ± 10 ppm and 1000 ± 10 ppm, respectively. As expected, the higher hypochlorite solution exhibited a stronger virucidal activity against FCV (Table 1). Similar results were previously reported when hypochlorite concentrations between 1000 and 5000 ppm were found to be virucidal against FCV. However, those studies were performed using a suspension test protocol with no soil.

Based on initial and confirmatory testing results, formulation R-82, a QAC disinfectant cleaner, demonstrated a log reduction against FCV that exceeded the EPA requirements for virucidal claims. Previous studies by Gulati et al. reported the inactivation of FCV on stainless steel surfaces using a QAC formulation in combination with sodium carbonate. However, in this study, the QAC formulation did not have any other active ingredient. Other studies reported the lack of virucidal activity against FCV by QAC-based disinfectants. However, the results of this study, which used FCV as a surrogate of NV demonstrated the effectiveness of formulation R-82 against FCV. Why was formulation R-82 efficacious against FCV when other QAC-based formulations did not show virucidal activity? Formulation R-82 contains a unique combination of dialkyl dimethyl ammonium chloride and alkyl dimethyl benzyl ammonium chloride called Bardac 208M (Lonza, Inc). The final QAC concentration in the use dilution tested was 850 ppm. Therefore, the high concentration of the unique QAC combination along with the high alkaline pH of the formulation might have provided a synergistic effect, increasing its virucidal activity against FCV.

The high virus titer obtained in the study, eg, 7.8 log$_{10}$, exceeds the real virus concentration in most environments. Norovirus is known to be highly infectious at much lower concentrations. As few as 100 virus particles can cause infection. Therefore, testing conditions in this study were extremely rigorous and stringent. Stringent disinfection procedures are needed to reduce the morbidity and transmission of NV because the virus remains viable on surfaces for a long time and is resistant to drying. Thorough disinfection procedures eliminate the possibility of outbreak spreading. Proper disinfection of contaminated hard surfaces with formulation R-82 entails saturating surfaces with the use dilution for 10 minutes, resulting in virus elimination and optimization of infection control practices by reducing the virus potential for spreading. For instance, it has been shown that disinfection and cleaning of contaminated surfaces with a combination of sodium hypochlorite and detergents reduced the spread of NV. However, the disinfection efficiency of hypochlorite might be reduced by the

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**Table 2. Virucidal effectiveness results of samples and controls for confirmatory testing**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Samples treated with R-82 disinfectant*</th>
<th>Samples treated with NaOCl† (100 ± 10 ppm)</th>
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The plus and minus signs represent 4 replicas.

ND, not determined; NaOCl, sodium hypochlorite; +, feline calicivirus-infected cells detected; cytotoxic effects observed; −, feline calicivirus-infected cells not detected; no cytotoxic effects observed; 0, no cytotoxicity observed.

*Averages of 3 batches performed in duplicates.

†Averages of duplicates.
presence of organic matter.

Sodium hypochlorite is also corrosive and must be removed from surfaces by a water rinse. When fingers come in contact with contaminated surfaces, NV is transferred to other surfaces and consequently to other people. Environmental sampling during outbreaks of NV conclusively demonstrated that transfer occurred on hand contact and other surfaces. Evidently, proper cleaning and disinfection of surfaces and environmental samples have provided an effective strategy to prevent the transmission of the NV from contaminated surfaces to hands, cloths, and other secondary surfaces.

In conclusion, this study demonstrates the suitability of a QAC formulation, R-82, to disinfect environmental surfaces contaminated by FCV, a surrogate of NV, after a 10-minute contact time at a temperature of 20°C ± 2°C. The choices for efficacious disinfectants against FCV and NV were limited to hypochlorite, phenolic-based formulations, and a peroxygen compound. This study expands the choices for infection control professionals to a QAC-based formulation, R-82.

References