ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2006, p. 1419–1424 Vol. 50, No. 4
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.4.1419–1424.2006

Efficacy of Hospital Germicides against Adenovirus 8, a Common Cause of Epidemic Keratoconjunctivitis in Health Care Facilities

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Received 17 August 2005/Returned for modification 17 October 2005/Accepted 6 February 2006

The inactivation of virus-contaminated nonporous inanimate surfaces was investigated using adenovirus type 8, a common cause of epidemic keratoconjunctivitis. A 10-μl inoculum of adenovirus was placed onto each stainless steel disk (1-cm diameter), and the inoculum was allowed to air dry for 40 min. Twenty-one different germicides (including disinfectants and antiseptics) were selected for this study based on their current uses in health care. After a 1- or 5-minute exposure to 50 μl of the germicide, the virus-germicide test mixture was neutralized and assayed for infectivity. Using an efficacy criterion of a 3-$\log_{10}$ reduction in the titer of virus infectivity and regardless of the virus suspending medium (i.e., hard water, sterile water, and hard water with 5% fetal calf serum), only five disinfectants proved to be effective against the test virus at 1 min: 0.55% *ortho*-phthalaldehyde, 2.4% glutaraldehyde, 2.65% glutaraldehyde, ~6,000 ppm chlorine, and ~1,900 ppm chlorine. Four other disinfectants showed effectiveness under four of the five testing conditions: 70% ethanol, 65% ethanol with 0.63% quaternary ammonium compound, 79.6% ethanol with 0.1% quaternary ammonium compound, and 0.2% peracetic acid. Of the germicides suitable for use as an antiseptic, 70% ethanol achieved a 3-$\log_{10}$ reduction under four of the five test conditions. These results emphasize the need for proper selection of germicides for use in disinfecting noncritical surfaces and semicritical medical devices, such as applanation tonometers, in order to prevent outbreaks of epidemic keratoconjunctivitis.

Adenoviruses are transmitted by direct contact, indirect contact via contaminated medical devices, small-droplet aerosols, the fecal-oral route, and occasionally, ingestion of contaminated water (16). A common adenoviral illness and the one most frequently associated with nosocomial outbreaks is epidemic keratoconjunctivitis (EKC). EKC has been most commonly associated with adenovirus types 8 and 19 but has also been reported with other serotypes, including types 2 to 4, 11, 14, 16, and 29. All types cause similar clinical syndromes, but types 8 and 19 are much more likely to be involved in large epidemics. Ocular symptoms include a foreign body sensation, photophobia, lacrimation, and intense conjunctivitis. In most cases, the infection remains self-limited and the patient's eyesight is unaffected (6).

Large outbreaks of epidemic keratoconjunctivitis have occurred in medical facilities. When adenoviral outbreaks occurred in health care facilities, attack rates reached as high as 25% (6). EKC outbreaks not only are common in eye clinics and hospitals but have also been documented in industrial plants, nursing homes, camps, military bases, and child care centers. The major modes of transmission are person to person via the hands of medical caregivers and ophthalmic instruments (e.g., tonometers and slit lamps) or contaminated ophthalmic solutions (e.g., wash stations and topical anesthetic solutions) (16). Infected health care workers may serve as both a reservoir for infection and a means of transmission of infection to other patients (6).

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Adenovirus is extremely hardy when deposited on environmental surfaces and may be recovered from plastic and metal surfaces for more than 30 days (7, 10). Thus, the elimination of adenovirus from inanimate surfaces and ophthalmic instruments is essential in preventing outbreaks of epidemic keratoconjunctivitis. Unfortunately, only limited data on the efficacy of available germicides against adenoviruses in general are available (9, 11, 18, 20), with only one report that evaluated adenovirus type 8 using only a single germicide (i.e., povidone-iodine) (20). Thus, we undertook this study to assess the efficacy of 21 different germicides against adenovirus type 8. Both disinfectants and antiseptics were tested.

MATERIALS AND METHODS

Viruses and cells. Adenovirus type 8 (ATCC strain VR-1085AS/RB) was obtained from the American Type Culture Collection (ATCC, Manassas, VA). To prepare the adenovirus for experimental use, it was propagated to increase the titer and then extracted. The propagation and extraction procedure has been described in detail elsewhere (11a).

Adenovirus type 8 was propagated and assayed in A549 cells. These A549 cells were cultured and maintained in Eagle's minimal essential medium containing 5% fetal calf serum. The adenovirus was grown and assayed by the liquid culture technique in confluent layers of A549 cells. The culture plates were four-row, 24-well plates. The titer of infectivity of adenovirus type 8 was estimated by a quantal analysis method based on the number of infected wells as determined from the observation of cytopathic effects (CPE) on inoculated cell cultures, according to the method developed by Reed and Muench (12).

Viral inactivation tests. The disk-based quantitative carrier test method was used to assess the virucidal activity of chemical germicides per the method of Sattar et al. (17). The procedure used is presented in Fig. 1. In brief, the method used brushed 4 stainless steel disks (1 cm in diameter) as carriers (Muzeen and Blythe Ltd., Winnipeg, Manitoba, Canada). Ten microliters of the test virus was placed on each disk, and the inoculum was dried under ambient conditions. The

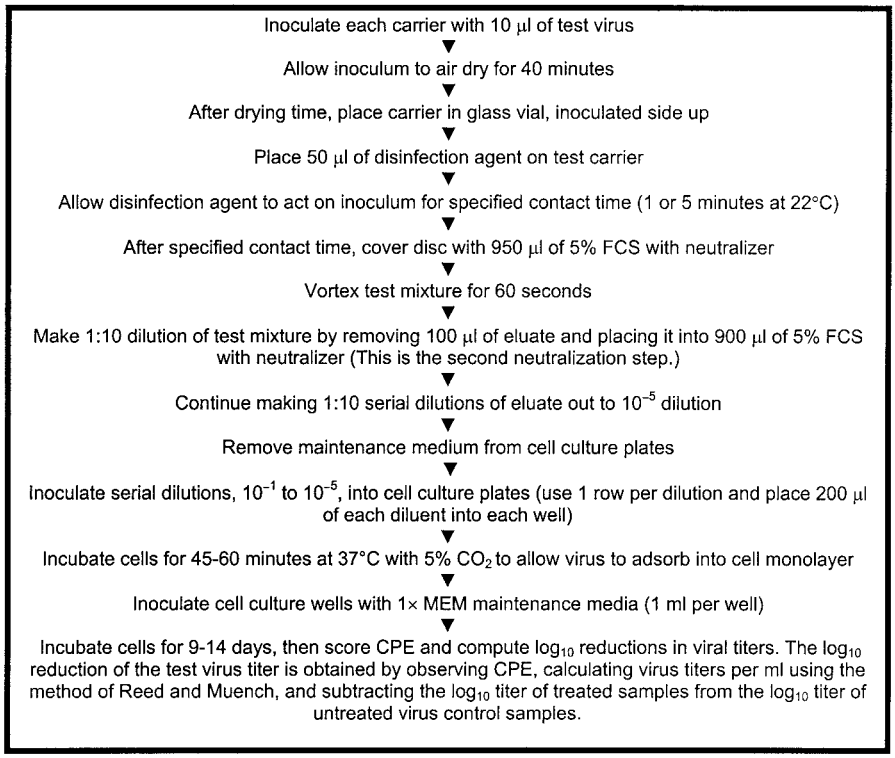

FIG. 1. Quantitative carrier test for virucidal activity protocol. FCS, fetal calf serum; MEM, minimum essential medium.

dried inoculum was then exposed to 50 μl of the test germicide or a control solution for a defined contact time (1 min or 5 min) at room temperature (~22°C). By direct observation, the 50-μl volume of germicide covered the entire dried virus inoculum. Five percent fetal calf serum with neutralizer (0.95 ml) was added to each carrier holder to dilute/neutralize the germicide; the inoculum was eluted, and the eluates were titrated in cell cultures to determine the degree of loss in virus viability. We determined the $\log_{10}$ reduction of the test virus by observing cytopathic effects in a liquid culture assay.

Germicides. Twenty-one different germicide types were tested: 12 disinfectants (10 low- and intermediate-level disinfectants, 2 high-level disinfectants), 3 chemical sterilants, 4 antiseptics, and 2 germicides used as both disinfectants and antiseptics (Table 1).

All germicides requiring dilution were diluted in "hard water" according to the manufacturer's recommendations. The use of hard water prevents variations in experimental results because of changes in tap water quality. The hard water was prepared according to the U.S. Environmental Protection Agency, Office of Pesticides Program protocol (L. Samalot, personal communication) (23), and provides an approximate hardness of 400 ppm. A water hardness of 380 to 420 ppm as calcium carbonate ($CaCO_3$) is considered to be standard hard water for use in disinfectant solutions. The hardness was determined using a hardness test kit (HACH Company, Loveland, CO), and the average water hardness was 415 ppm. The sterile water used was Aqualite (Hospira Inc., Lake Forest, IL). This product is sterile, distilled, nonpyrogenic water.

All germicides were made according to the manufacturer's instructions, and all products were tested within the manufacturer's use life. For evaluation of the Steris system disinfectant, the contents of the Steris carton were diluted into 6.1 liters of hard water. The mixture was stirred until all contents were completely dissolved as has previously been described (2), and then the sterilant was immediately used in the assay. Chlorine determinations were done using an *N*,*N*-diethyl-*p*-phenylenediamine titration with ferrous ammonium sulfate and represent free chlorine residual (8). The germicides were stored at room temperature, and those requiring dilution were made fresh each day no more than 3 hours prior to use in experimental trials.

Neutralization. Arresting the virucidal activity of the germicide immediately at the end of the contact time is critical in generating meaningful results (17). In this study, neutralization was achieved by both dilution and chemical neutralization. First, after the contact time, 950 μl of 5% fetal calf serum plus chemical neutralizers was added to the well, and the solution was vortexed for 60 seconds. Second, after the virus-germicide mixture had been chemically neutralized, 100 μl of the virus-germicide mixture was placed into 900 μl of 5% fetal calf serum with neutralizers (i.e., a 1:10 dilution). Three percent glycine was the neutralizer used for all the germicides except 4% chlorhexidine gluconate (BactoShield), Sterilox (218 and 695 ppm chlorine), and ~1,900 ppm chlorine (Clorox Clean-up). Sterilox was provided premade by the manufacturer and stored at refrigerator temperatures in a closed, brown plastic container, and the indicated chlorine concentrations were obtained after use. Sodium thiosulfate (0.1%) was used to neutralize 4% chlorhexidine gluconate and ~1,900 ppm chlorine, because cytotoxicity was observed when 3% glycine was used.

Controls. The objective of the cytotoxicity control was to determine the dilution of the germicide that causes no cytotoxicity of the cell line used to measure virus infectivity and assess whether the neutralizer reduces or enhances such cytotoxicity. This cytotoxicity testing was done as recommended by Sattar et al. (17), by making an initial 1:20 dilution and one further 10-fold dilution of the used dilution of the germicide in 5% fetal calf serum with and without neutralizers. The culture medium from the A549 cells was removed, and 100 μl of the diluted inoculum was added to each well of A549 cells and incubated for 45 to 60 min at 37°C with 5% CO_2. Control monolayers received 100 μl of only 5% fetal calf serum with neutralizers. After this time, culture medium was reapplied to the A549 cells, and the cells were incubated for 7 to 12 days and monitored for any visible cytotoxicity. Results demonstrated that the test formulations had no significant cytotoxic effects.

Levels of the test substance which show no obvious cytotoxicity could reduce or enhance the ability of adenovirus to infect or replicate in the host cells, thus interfering with the estimation of its virucidal activity (17). An interference with virus infectivity control was conducted per the methodology described by Sattar et al. (11a, 17). There was no significant interference with any germicide.

Statistical analysis. Mean $\log_{10}$ reduction in viral titer was calculated, and germicides were classified according to efficacy of disinfection. Then, a two-way analysis of variance was performed to assess the relative impact of germicides and test conditions of $\log_{10}$ reductions in viral titer. This analysis focused primarily on differences between test conditions after controlling for the germicide.

TABLE 1. Germicides tested and their active ingredients and test concentrations

Germicide name	Manufacturer and location	Active ingredient(s)	Formulation(s) tested	Classification(s)
Steris 20 sterilant	Steris Corp., Mentor, OH	35% Peracetic acid	0.2%	Chemical sterilant
Cidex OPA	Advanced Sterilization Products, Irvine, CA	0.55% *ortho*-phthalaldehyde	Undiluted	High-level disinfectant
Cidex	Advanced Sterilization Products, Irvine, CA	2.4% Glutaraldehyde	Undiluted	Chemical sterilant
Wavicide-01	Medical Chemical Corp., Torrance, CA	2.65% Glutaraldehyde	Undiluted	Chemical sterilant
Clorox	Clorox Company, Oakland, CA	6% Sodium hypochlorite	1:10 (~6,000 mg/liter) and 1:50 (~1,200 mg/liter) dilutions	Disinfectant
Clorox Clean-up cleaner	Clorox Company, Oakland, CA	1.84% Sodium hypochlorite	Undiluted (~1,910 mg/liter)	Disinfectant
Vesphene II se	Steris Corp., St. Louis, MO	9.09% *o*-phenylphenol, 7.66% *p*-tertiary amylphenol	1:128 dilution	Disinfectant
Isopropyl alcohol	HUMCO, Texarkana, TX	70% Isopropyl alcohol	Undiluted	Disinfectant
Ethanol	Acros Organics, Fair Lawn, NJ	70% Ethanol	Undiluted	Disinfectant
Hydrogen peroxide	Bergen Brunswig Drug Co., Orange, CA	3% Hydrogen peroxide	Undiluted	Disinfectant
Clorox disinfectant spray	Clorox Company, Oakland, CA	65% Ethanol, 0.63% QAC[a]	Undiluted	Disinfectant
Lysol brand II disinfectant spray	Reckitt Benckiser, Wayne, NJ	79.6% Ethanol, 0.1% QAC[b]	Undiluted	Disinfectant
TBQ	Steris Corp., St. Louis, MO	8% QAC[c]	1:128 dilution	Disinfectant
Novaplus	Novation, Irving, TX	10% Povidone-iodine (1% titratable iodine)	Undiluted	Antiseptic
Dettol	Reckitt Benckiser, Hull, United Kingdom	4.8% Chloroxylenol	1:20 and 1:40 dilutions	Antiseptic/disinfectant
BactoShield	Steris Corp., St. Louis, MO	4% Chlorhexidine gluconate	Undiluted	Antiseptic/surgical hand scrub
Medicated Soft 'N Sure	Steris Corp., St. Louis, MO	0.5% Triclosan	Undiluted	Antiseptic/handwash
Acute-Kare	Steris Corp., St. Louis, MO	1% Chloroxylenol	Undiluted	Antiseptic/handwash
Accel TB	Virox, Oakville, Ontario, Canada	0.5% Accelerated hydrogen peroxide	Undiluted	Disinfectant
Microcyn	Oculus, Petaluma, CA	~80 mg/liter chlorine	Undiluted	Disinfectant/antiseptic
Sterilox	Sterilox Technologies, Radnor, PA	Chlorine (superoxidized water)	Undiluted (~170 ppm and ~640 ppm)	High-level disinfectant

[a] 0.63% QAC, quaternary ammonium compound containing 0.1890% octyl decyl dimethyl ammonium chloride, 0.0945% dioctyl dimethyl ammonium chloride, 0.945% didecyl dimethyl ammonium chloride, and 0.2520% alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium chloride.
[b] 0.1% QAC, quaternary ammonium compound containing alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium saccharinate.
[c] 8% QAC, quaternary ammonium compound containing alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium chloride.

RESULTS

The experimental data for inactivation of adenovirus type 8 by the germicides tested are shown in Table 2. These data show the mean $\log_{10}$ reductions in viral titer over multiple independent trials ($n = 2$ to 5 trials), using various exposure times (i.e., 1 and 5 min) and test conditions (i.e., sterile water, hard water, and hard water plus 5% fetal calf serum). Germicides that demonstrated at least a 3-$\log_{10}$ reduction in the titer of adenovirus type 8 using the most challenging test conditions (i.e., 5% fetal calf serum plus hard water and a 1-min exposure time) and thus were considered to be effective germicides for the elimination of adenovirus type 8 dried on environmental surfaces/medical devices included 0.55% *ortho*-phthalaldehyde (Cidex OPA), 0.2% peracetic acid (Steris 20 sterilant), 2.4% glutaraldehyde (Cidex), 2.65% glutaraldehyde (Wavicide-01), ~6,000 ppm chlorine (1:10 dilution of Clorox), ~1,900 ppm chlorine (Clorox Clean-up), and 79.6% ethanol with 0.1% quaternary ammonium compound (Lysol disinfectant spray). All of these germicides were also effective at 5 min except ~6,000 ppm chlorine. It is unclear as to why the 6,000 ppm chlorine was effective at 1 min (~4-$\log_{10}$ reduction) but produced only an ~1.5-$\log_{10}$ reduction at 5 min. Cidex (2.4% glutaraldehyde) was not tested at 5 min.

Germicides exhibiting at least a 3-$\log_{10}$ reduction in the titer of adenovirus type 8, using the most challenging test condition of hard water and 5% fetal calf serum and a 5-min contact time but less than a 3-$\log_{10}$ reduction in viral titer with a 1-min contact time, were 70% ethanol and 65% ethanol with 0.63% quaternary ammonium compound (Clorox disinfectant spray).

Germicides that did not demonstrate at least a 3-$\log_{10}$ reduction in titers of adenovirus type 8 with either a 1- or a 5-min contact time and under any test conditions (including sterile water) and thus were considered to be ineffective against adenovirus type 8 were as follows: 3% hydrogen peroxide, 0.0625% quaternary ammonium compound (TBQ; 1:128 dilution of 8%), 0.13% phenolic (Vesphene II se; 1:128 dilution of 16.72%), 70% isopropyl alcohol, 10% povidone-iodine (Novaplus), 0.24% and 0.12% chloroxylenol (Dettol; 1:20 and 1:40 dilution of 4.8% chloroxylenol), 4% chlorhexidine gluconate, 0.5% triclosan (Medicated Soft 'N Sure), 1% chloroxylenol (Acute-Kare), 0.5% accelerated hydrogen peroxide (Accel TB), ~80 ppm chlorine

TABLE 2. Effectiveness of 21 germicides against adenovirus 8 under various test conditions

Germicide	Mean $\log_{10}$ reduction under test condition (no. of trials)[a]				
	Hard water for 1 min (4)	Hard water for 5 min (5)	Hard water + 5% fetal calf serum for 1 min (2)	Hard water + 5% fetal calf serum for 5 min (2)	Sterile water for 1 min (2)
Cidex OPA (*ortho*-phthalaldehyde)	4.37	5.65[b]	3.65	2.97	4.84
TBQ (quaternary ammonium compound)	0.38	0.33	0.72	0.30	0.17
Steris (peracetic acid)	2.50	4.75[b]	3.90	3.63	4.00
Vesphene II se (phenolic)	0.41	0.75	0.09	0.30	0.67
Cidex (glutaraldehyde)	4.87	NT	5.30	NT	4.84
Isopropyl alcohol (70%)	0.95	0.74	0.47	0.48	1.09
Wavicide-01 (glutaraldehyde)	4.87	NT	3.97	5.47	4.84
Hydrogen peroxide (3%)	0.43	0.36	0.22	0.30	0.44
Chlorhexidine (4%)	0.66	1.13	0.22	0.30	0.00
Clorox (1:50)	1.99	3.67	1.22	0.72	0.85
Clorox (1:10)	4.87	NT	3.97	1.48	4.84
Ethanol (70%)	4.62	4.33	1.97	5.47	3.67
Medicated Soft 'N Sure	0.18	0.20	0.55	0.30	0.17
Acute-Kare	0.38	0.24	0.05	0.40	0.17
Povidone-iodine (10%)	0.91	0.76	0.42	0.48	1.17
Lysol disinfectant spray	3.53	4.33	4.05	5.47	2.85
Clorox disinfectant spray	4.87	4.46	0.82	5.47	4.00
Clorox Clean-up	4.45	5.32[b]	4.15	3.97	4.84
Dettol (1:20)	0.66	0.41	0.00	0.30	0.19
Dettol (1:40)	0.55	0.36	0.40	0.30	0.17
Accel TB	0.67[b]	0.57[b]	0.40	0.48	NT
Microcyn	0.65[b]	0.25[b]	0.30	0.40	NT
Sterilox (218 ppm chlorine)	0.67[b]	0.35[b]	0.38	0.30	NT
Sterilox (695 ppm chlorine)	0.82[b]	2.98[b]	0.30	0.51	NT

[a] Values are $\log_{10}$ viral titer reductions. NT, not tested. The viral carrier quantitation means were $10^{4.9}$ for hard water for 1 min, $10^{4.5}$ for hard water for 5 min, $10^{5.3}$ for 5% fetal calf serum and hard water for 1 min, $10^{5.5}$ for 5% fetal calf serum and hard water for 5 min, and $10^{4.8}$ for sterile water for 1 min.
[b] Only two trials performed.

(Microcyn), and ~218 ppm chlorine. The latter three germicides were not tested with sterile water.

The estimated summary $\log_{10}$ reductions for the five test conditions were as follows: hard water with a 1-min contact time, 1.89; hard water with a 5-min contact time, 2.12; 5% fetal calf serum and hard water with a 1-min contact time, 1.37; 5% fetal calf serum and hard water with a 5-min contact time, 1.70; and sterile water, 1.71. In an analysis of variance, differences among disinfectants affected $\log_{10}$ kill much more than test conditions. While the r^2 for a model with disinfectant only was 0.797, the r^2 with disinfectant and a test condition improved only slightly to 0.812. A significantly higher $\log_{10}$ reduction was demonstrated for studies using hard water than for those using 5% fetal calf serum plus hard water. Specifically, a higher $\log_{10}$ reduction was demonstrated with hard water with a 1-min contact time than with 5% fetal calf serum and hard water with a 1-min contact time ($P = 0.0013$). Similarly, the $\log_{10}$ reduction with hard water with a 5-min contact time was significantly greater than that achieved with 5% fetal calf serum plus hard water for 5 min ($P = 0.0114$). No statistical difference was exhibited comparing hard water plus fetal calf serum for 1 min with sterile water, although there was a trend towards a lower $\log_{10}$ reduction in the presence of fetal calf serum and hard water ($P = 0.0771$). There was no statistical difference between 1- and 5-min contact times for either hard water alone or 5% fetal calf serum plus hard water, although with both comparisons, the longer contact time tended to produce a greater $\log_{10}$ reduction ($P = 0.1056$ for 1-min versus 5-min contact time for hard water only; $P = 0.0721$ for 1-min versus 5-min contact time for 5% fetal calf serum plus hard water). In two instances (i.e., 2.4% glutaraldehyde and ~6,000 ppm chlorine) in which excellent activity was demonstrated at 1 min, the germicide was not tested at 5 min. This presumably would have resulted in a higher mean $\log_{10}$ kill at 5 min, and this may have enhanced the likelihood of finding a statistical difference between the 1- and 5-min exposures in hard water.

The addition of organic matter (i.e., 5% fetal calf serum) into the viral suspension resulted in a decrease in the effectiveness of viral titer reduction of several germicide products at a contact time of 1 or 5 min. The viral titer was reduced by $\geq$1.0 to 4.0 $\log_{10}$ when adenovirus type 8 was in the presence of organic matter for the following germicides: 0.55% *ortho*-phthalaldehyde, 0.2% peracetic acid, ~1,200 ppm chlorine (1:50 dilution of Clorox), 70% ethanol, 65% ethanol with 0.63% quaternary ammonium compound, ~695 ppm chlorine, and ~1,900 ppm chlorine. This loss of virucidal activity in the presence of organic matter resulted in some germicides (i.e., ~1,200 ppm chlorine at 5 min, 70% ethanol at 1 min, 65% ethanol with 0.63% quaternary ammonium compound at 5 min, and ~695 ppm chlorine at 5 min) being reclassified as ineffective.

DISCUSSION

Adenovirus type 8 is extremely hardy when deposited on environmental surfaces and inanimate objects, thus explaining why fomites and medical equipment play such an important role in nosocomial transmission (6, 7, 10). Fifty percent of infected patients are found to have adenovirus type 8 on their hands, and adenovirus can be recovered from metal and plastic surfaces for more than 30 days (1, 6, 7). To prevent the spread

of adenovirus, the Centers for Disease Control and Prevention (3) and the Association for Professionals in Infection Control and Epidemiology (13) have recommended that tonometer tips be cleaned with soap and water and then disinfected by soaking them for 5 to 10 min in a solution containing either 5,000 ppm chlorine, 3% hydrogen peroxide, 70% ethyl alcohol, or 70% isopropyl alcohol. However, there is only one study available on the efficacy of a single germicide against adenovirus 8 (20). This knowledge deficit about the efficacy of germicides for the eradication of adenoviruses was the reason for this study.

The test protocol used allows the germicide activity to be determined by simulating the drying of a viral agent onto an environmental surface, followed by treatment with various germicidal products (17). We determined the $\log_{10}$ reduction of the test virus by observing CPE in a liquid culture assay. The viral titer was expressed using the method of Reed and Muench (12). This experimental protocol was chosen because carrier testing is believed to produce results similar to those actually encountered in health care settings, as opposed to suspension testing, whose results are believed to be less applicable to actual clinical practice (17). This is because viral susceptibility to germicides is dependent upon whether a virus is wet or dried. Sattar and coworkers found that a number of test disinfectants effectively reduced the titer of rotavirus in suspension testing, yet had no effect in the carrier test when the virus was dried on a nonporous surface (19). Since, in health care settings, microorganisms are adherent to surfaces or imbedded in debris, the carrier test can better simulate actual in-use situations and thus produce more reliable data (17). The method of assessing the germicidal efficacy is the reduction in viral titer. No international standard for viral titer reduction for germicide product effectiveness has been established; however, Sattar et al. state that for viruses, a 2- to 4-$\log_{10}$ reduction in titer on hard surfaces is the usual objective (17). In this study, while it is not known what level of viral reduction is needed to prevent adenovirus transmission, an "effective" germicide provided at least a 3-$\log_{10}$ reduction in the titer of adenovirus.

Two different contact times were used in this study, 1 min and 5 min. A 1-min contact time was chosen as this is the normal drying time in which a disinfectant is applied to a noncritical environmental surface (e.g., countertop) with a wet cloth, and a 5-minute contact time was chosen as this is the minimum time recommended by CDC guidelines for disinfecting applanation tonometers (3). Using an efficacy criterion of a 3-$\log_{10}$ reduction in the titer of virus infectivity and regardless of the virus suspending medium (i.e., hard water, sterile water, and hard water with 5% fetal calf serum), only four disinfectants proved to be effective against the test virus at all tested contact times (1 and/or 5 min), and they were 0.55% *ortho*-phthalaldehyde, 2.4% glutaraldehyde, 2.65% glutaraldehyde, and ~1,900 ppm chlorine. Five other germicides showed effectiveness at all but one of the testing conditions (e.g., four out of five testing conditions, three out of four testing conditions): 65% ethanol with 0.63% quaternary ammonium compound, ~6,000 ppm chlorine, 70% ethanol, 79% ethanol with 0.1% quaternary ammonium compound, and 0.2% peracetic acid. Of the germicides suitable for use as an antiseptic, only 70% ethanol achieved a 3-$\log_{10}$ reduction under most test conditions (four of five test conditions). These results are consistent with those of other investigators when they tested other serotypes of adenoviruses (9, 18). An important finding from our study was that of the four disinfectants recommended by the CDC and Association for Professionals in Infection Control and Epidemiology for elimination of adenovirus type 8 from ophthalmic instruments, two (70% isopropyl alcohol and 3% hydrogen peroxide) were found to be ineffective. Based on these data, 3% hydrogen peroxide and 70% isopropyl alcohol are not effective against adenovirus that is capable of causing epidemic keratoconjunctivitis and similar viruses and should no longer be used for disinfecting applanation tonometers. These results emphasize the proper selection of disinfectants for use in disinfecting semicritical medical devices, such as applanation tonometers.

The literature also states that sometimes 70% isopropyl alcohol wipes are used in the disinfection of tonometer tips because a short and simple disinfection procedure is desired (5). Craven et al. suggest that this disinfection technique may prove effective in adenovirus elimination (5); however, the viral inoculum was very low. Once again, the results of our study dispute this recommendation, as 70% isopropyl alcohol was found to be ineffective in eliminating or appreciably reducing adenovirus type 8.

Only 0.55% *ortho*-phthalaldehyde, 2.4% glutaraldehyde, 2.65% glutaraldehyde, 70% ethyl alcohol, 0.2% peracetic acid, and a 1:10 dilution of household bleach containing about 6,000 ppm chlorine could be used to disinfect applanation tonometers, provided that the device manufacturer ensures material compatibility. In general, only products registered by the Food and Drug Administration as high-level disinfectants and/or chemical sterilants are used for disinfection of medical devices in contact with mucous membranes (15). Thus, products such as 65% ethanol with 0.63% quaternary ammonium compound and 79% ethanol with 0.1% quaternary ammonium compound are not used for high-level disinfection of semicritical items. Another consideration when choosing one of the recommended germicides is the effective wash-off of these germicides from the tonometer tips. This is important because tonometers are in contact with the cornea and germicide residue from some products, such as aldehydes, and could potentially injure the conjunctiva, causing an adverse health outcome. In addition, structural damage to Schiotz tonometers has been observed with 1:10 sodium hypochlorite (5,000 ppm chlorine) (4). For these reasons, after disinfection, the tonometer should be thoroughly rinsed in tap water and air dried before use.

Since adenovirus is stable on noncritical environmental surfaces, the surfaces must be disinfected with an effective disinfectant to prevent the transfer of virus from hand to surface to patient. The disinfectants that should be used for effective surface disinfection include effective products, such as ~1,900 ppm available free chlorine, 65% ethanol with 0.63% quaternary ammonium compound, 79.6% ethanol with 0.1% quaternary ammonium compound, and 70% ethanol. These disinfectants should be allowed to contact all environmental surfaces for at least a minute (applied wet and allowed to dry), ensuring that maximal reduction in titers of adenovirus occurs. Since high-level disinfectants and chemical sterilants, such as glutaraldehyde, *ortho*-phthalaldehyde, and peracetic acid, are not recommended for use on noncritical instruments and devices or any environmental surfaces, these products should not be used for noncritical items or surfaces (21).

An unanticipated finding was that the efficacy of the test

germicide products was not significantly affected when the experimental variable of water quality for germicide preparation was varied. The water quality variables examined were preparation of the germicides with hard water (380 to 420 ppm as $CaCl_2$) versus sterile water. When sterile water was used instead of hard water, germicide efficacy was unaffected. When the viral particles were in the presence of an organic load (modeled with 5% fetal calf serum) and hard water, as is commonly found in health care settings, the effectiveness of the germicide was significantly affected. The virucidal activity of these germicides (i.e., Cidex OPA, 0.2% peracetic acid, Clorox [diluted 1:50], 70% ethanol, Clorox disinfectant spray, Clorox Clean-up, and ~695 ppm Sterilox) was reduced by >1 to 4 $\log_{10}$ when adenovirus type 8 was in the presence of organic matter. These results are consistent with other studies that demonstrate reduced effectiveness of chlorine and alcohol in the presence of organic matter, as the organic matter may protect adenoviruses from exposure or change the virucidal properties of the germicide (14, 24). This loss of virucidal activity resulted in some germicides (i.e., Clorox [1:50] at 5 min, ethanol at 1 min, Clorox disinfectant spray at 1 min, and Sterilox at 5 min) being reclassified as ineffective.

In summary, several germicides (peracetic acid, aldehydes [glutaraldehyde and *ortho*-phthalaldehyde], chlorine-based products [1,900 to 6,000 ppm available free chlorine], ethyl alcohol, and ethanol mixed with quaternary ammonium compounds) produced a 3- to 4-$\log_{10}$ reduction in the titer of adenovirus type 8 with a contact time of 1 and/or 5 min. We recommend that ophthalmologic equipment be disinfected with 70% ethyl alcohol or ~5,000 ppm chlorine (presuming compatibility with instruments). High-level disinfectants (e.g., 0.55% *ortho*-phthalaldehyde, ≥2.4% glutaraldehyde, and 0.2% peracetic acid) are also effective at killing adenovirus but must be compatible with the instrument and rinsed thoroughly with water to prevent eye damage. Environmental surfaces should be disinfected with effective products, such as ~1,900 chlorine, 65% ethanol with 0.63% quaternary ammonium compound, and 79% ethanol with 0.1% quaternary ammonium compound. Hand hygiene should be accomplished with an antimicrobial soap and water when adenoviral contamination may have occurred, until alcohol-based hand rubs are shown effective in human challenge studies (22).

ACKNOWLEDGMENTS

We express our appreciation to Gwy-Am Shin and Syed A. Sattar for technical guidance, Lisa M. Casanova for the chlorine determinations, and Gregory P. Samsa for statistical analysis. We also thank S. A. Sattar for supplying the stainless steel disks, Virox for supplying the Accel TB, Oculus for supplying the Microcyn product, and Sterilox for supplying the Sterilox product. W.A.R. has provided consultation to Clorox and Advanced Sterilization Products, and D.J.W. has provided consultation to Clorox.

This study was supported in part by the North Carolina Statewide Program for Infection Control and Epidemiology and the University of North Carolina Hospitals.

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