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Chemical Disinfection To Interrupt Transfer of Rhinovirus Type 14 from Environmental Surfaces to Hands

SYED A. SATTAR,[1]* HEATHER JACOBSEN,[1] V. SUSAN SPRINGTHORPE,[1] TIMOTHY M. CUSACK,[2] AND JOSEPH R. RUBINO[2]

Department of Microbiology & Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5,[1] and L&F Products, Montvale, New Jersey 07645[2]

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Rhinoviruses can survive on environmental surfaces for several hours under ambient conditions. Hands can readily become contaminated after contact with such surfaces, and self-inoculation may lead to infection. Whereas hand washing is crucial in preventing the spread of rhinovirus colds, proper disinfection of environmental surfaces may further reduce rhinovirus transmission. In this study, the capacities of Lysol Disinfectant Spray (0.1% *o*-phenylphenol and 79% ethanol), a domestic bleach (6% sodium hypochlorite diluted to give 800 ppm of free chlorine), a quaternary ammonium-based product (7.05% quaternary ammonium diluted 1:128 in tap water), and a phenol-based product (14.7% phenol diluted 1:256 in tap water) were compared in interrupting the transfer of rhinovirus type 14 from stainless steel disks to fingerpads of human volunteers upon a 10-s contact at a pressure of 1 kg/cm^2. Ten microliters of the virus, suspended in bovine mucin (5 mg/ml), was placed on each disk, and the inoculum was dried under ambient conditions; the input number on each disk ranged from 0.5 × 10^5 to 2.1 × 10^6 PFU. The dried virus was exposed to 20 μl of the test disinfectant. The Lysol spray was able to reduce virus infectivity by >99.99% after a contact of either 1 or 10 min, and no detectable virus was transferred to fingerpads from Lysol-treated disks. The bleach (800 ppm of free chlorine) reduced the virus titer by 99.7% after a contact time of 10 min, and again no virus was transferred from the disks treated with it. On the other hand, the quaternary ammonium and phenolic products were able to inactivate only 14.7 and 62.3% of the virus on the disks, respectively; contact of fingerpads with disks treated separately with these products resulted in the transfer of 8.4% ± 3.6 and 3.3% ± 1.9%, respectively, of the infectious virus remaining on the disks after the disinfectant was allowed to dry. Virus transfer from the control disks was 0.58% ± 0.35%. These findings suggest that care must be exercised in the selection of disinfectants if transfer of rhinoviruses from environmental surfaces to human hands is to be interrupted efficiently.

Nearly two decades ago, Hendley et al. (20) showed that rhinoviruses could survive on human hands for several hours and that self-inoculation by rubbing of the nasal mucosa or conjunctivae with virus-contaminated fingers could lead to infection in susceptible hosts. These findings were subsequently confirmed and extended (6, 14, 21, 33). It has also been demonstrated that hand antisepsis can reduce the transmission of rhinovirus colds (15, 17, 18).

Environmental surfaces become contaminated with rhinoviruses through contact with contaminated hands (3, 20) and by the settling of aerosolized nasopharyngeal secretions (35). Rhinoviruses can also survive for several hours on a variety of nonporous materials (3, 20, 32, 33, 37), and infectious viruses have been recovered from naturally contaminated objects in the surroundings of persons with rhinovirus colds (33). Clean hands can readily pick up rhinoviruses by touching or handling such objects (3, 20, 31, 33), and hands contaminated in this way can also subsequently spread the infection (12). This suggests that decontamination of hands alone may not be sufficient to prevent the spread of rhinovirus colds and that, where appropriate, such measures should be instituted in conjunction with the proper and regular disinfection of environmental surfaces as well. Whereas the findings of preliminary studies have shown this approach to be promising (12, 15), no systematic and quantitative investigations have been undertaken in this regard. The present study was designed to address this question by first comparing the rhinovirucidal activity of four commercially available products and then testing how effectively their application on nonporous environmental surfaces could interrupt the transfer of rhinovirus type 14 to human hands.

* Corresponding author.

MATERIALS AND METHODS

Cells. A seed culture of the A-5 strain of HeLa cells was kindly provided to us by B. Korant of E. I. du Pont de Nemours & Co., Wilmington, Del. In our laboratory the cells were routinely grown in 75-cm^2 plastic flasks (Costar, Cambridge, Mass.) in Eagle minimum essential medium supplemented with 2 mM L-glutamine and 0.2% sodium bicarbonate; antibiotics were added to the medium to give a final concentration (per milliliter) of 100 μg of kanamycin, 100 U of penicillin G (sodium salt), 100 μg of streptomycin sulfate, and 0.25 μg of amphotericin B. The growth medium also contained 7% heat-inactivated (56°C for 30 min) fetal bovine serum. The medium and all the supplements were purchased from GIBCO (Grand Island, N.Y.). The methods for culturing and passaging these cells have already been described in detail (23).

Virus. Strain 1059 of human rhinovirus type 14 was obtained from J. Gwaltney, Jr. (University of Virginia, Charlottesville). The virus was plaque purified once by us in A-5 HeLa cells, and its identity was further confirmed through neutralization with a typing serum obtained from the National Institute of Allergy and Infectious Diseases, Be-

TABLE 1. Relevant information on the four environmental surface disinfectants tested in this study

Product	Formulation	Contact time (min)	Dilution tested
LDS (sold in a pressurized can as a ready-to-use spray)	*o*-Phenylphenol, 0.1%, and ethanol, 79%	10	Undiluted
Domestic bleach (sold as a liquid concentrate for one-step cleaning and disinfection)	Minimum 6% sodium hypochlorite	Not given	Diluted in tap water to give 500 to 800 ppm of free chlorine
A quaternary ammonium-based product (sold as a liquid concentrate for one-step cleaning and disinfection)	Alkyl (C_{14}, 50%; C_{12}, 40%; C_{16}, 10%) dimethyl benzyl ammonium chloride, 2.680%; octyl decyl dimethyl ammonium chloride, 2.010%; didecyl dimethyl ammonium chloride, 1.355%; and dioctyl dimethyl ammonium chloride, 1.005%	10	1:128 in tap water as recommended on product label to give about 550 ppm of quaternary
A phenol-based product (sold as a liquid concentrate for one-step cleaning and disinfection)	*o*-Phenylphenol, 7.3%, and *p*-tertiary amylphenol, 7.4%	10	1:256 in tap water as recommended on product label to give about 575 ppm of phenolic

thesda, Md. For the preparation of virus pools, the medium was removed from monolayers of HeLa cells in 75-cm^2 flasks, and each flask containing a monolayer was inoculated with 0.1 ml of the virus to give a multiplicity of infection of approximately 0.1; control cultures received the same volume of Earle balanced salt solution (EBSS; GIBCO). The inoculated monolayers were held at 33°C for 30 min to allow for virus adsorption. At the end of this period, 20 ml of supplemented Eagle minimum essential medium without serum was added to each flask, and they were placed back at 33°C. Within 36 h nearly 75% of each virus-inoculated monolayer showed cytopathology, and at this stage the flasks were frozen (−20°C) and thawed three times and the virus pool was aliquoted in 1.0-ml quantities in plastic vials for storage at −80°C. Just before an experiment, a vial was centrifuged (Sorval MC12V; Sorval-Du Pont, Newtown, Conn.) at 12,000 rpm for 3 min at 4°C in rotor F-12/M.18 to remove cellular debris, and the supernatant was used for the contamination of the disks. Three separate virus pools were used in this study, and the amount of infectious virus in them ranged from 0.7×10^7 to 2.0×10^8 PFU/ml.

Disinfectants. The U.S. formulation of LDS was the primary focus of this study. However, its performance against the rhinovirus was compared with that of three other commercially available products widely used for the disinfection of environmental surfaces. Relevant information on all four disinfectants is presented in Table 1. LDS, the quaternary, and the phenolic are registered with the U.S. Environmental Protection Agency (EPA).

The product lots tested were as sold in the open market. Two separate lots of LDS (B10453 NJ2, Original Scent; and IL 11141-2, Fresh Scent) and the bleach and one lot each of the other two products were assessed in this study. Once they were received in our laboratory, the containers of the four products were stored at room temperature where only authorized individuals had access to them. Immediately prior to each experiment, LDS was collected as a liquid by spraying the product directly into a clean and sterile 13-ml plastic screw-cap vial (Sarstedt, Ville St. Laurent, Quebec, Canada). The quaternary and the phenolic were first diluted in tap water according to the directions given on their respective labels. A freshly purchased bottle of the bleach was first tested for its free chlorine content by the *N,N*-diethyl-*p*-phenylene-diamine method (1) with a commercial kit (Hach Chemical Co., Ames, Iowa); the disinfectant was then diluted in tap water and used at a free chlorine concentration of 500 to 800 ppm.

Plaque assays. For plaque assays, 12-well plastic plates (Costar) seeded with HeLa cells were incubated at 37°C in a 5% CO_2 atmosphere for about 48 h for monolayer formation. The growth medium was removed, and each monolayer was washed once with 2 ml of EBSS. Each well was then inoculated with 0.1 ml of an appropriate dilution (in EBSS) of the virus, by using at least three wells for every 10-fold dilution tested; cell culture controls received 0.1 ml of EBSS instead. The inoculated plates were held for approximately 1 h at 33°C in a 5% CO_2 atmosphere to allow for virus adsorption. At the end of this period, each monolayer was overlaid with 2.0 ml of supplemented Eagle minimum essential medium containing, in addition, 30 mM $MgCl_2$ (BDH, Toronto, Ontario, Canada), 50 μg of DEAE-dextran (Sigma Chemical Co., St. Louis, Mo.) per ml, 100 μg of 5-bromo-2′-deoxyuridine (Sigma) per ml, 2% fetal bovine serum, and 0.9% Oxoid agar 1 (Oxoid Ltd., Basingstoke, Hampshire, England). The plates with overlaid monolayers were sealed individually in clear plastic bags (Dazey Corp., Industrial Airport, Kans.) and incubated at 33°C for about 60 h. As described in detail before (36), the cultures were then fixed overnight in a 3.7% solution of formaldehyde in normal saline and stained for 1 min in a 1% aqueous solution of crystal violet for counting plaques, and the titer was expressed as PFU. The limit of detection of our plaque assay system was <10 PFU.

Virus-suspending media. Most of the experiments in this study were conducted with 5 mg of bovine mucin (product M-4503; Sigma) per ml as the virus-suspending medium in order to simulate human nasopharyngeal secretions (8). The mucin was first dissolved in sterile, deionized water to obtain a solution of 50 mg/ml, and before each experiment one part of it was mixed with nine parts of the virus. In certain experiments either nasal discharge, collected during an episode of acute rhinitis from an adult male volunteer, or 5% fetal bovine serum was used as the virus-suspending medium. The nasal discharge, which was used here without any centrifugation or filtration, was noninhibitory for the rhinovirus and did not induce any degeneration of A-5 HeLa cell

monolayers even after 5 days of incubation at 33°C. One part of the virus pool was suspended in nine parts of the nasal discharge just before the contamination of the disks.

Disks. Clean and sterile stainless disks (1-cm diameter), punched out of number 4 polished stainless steel sheets (0.75-mm thickness) purchased locally, were used as representative nonporous inanimate surfaces. The same disks were used repeatedly in this study. The used disks were first decontaminated by autoclaving them in a glass vial containing deionized water. They were then soaked overnight in a 2% solution of 7× detergent (Linbro; Flow Laboratories, McLean, Va.), thoroughly rinsed in deionized water, sterilized by autoclaving, and dried in a hot-air oven. During an experiment the disks were handled only with sterile forceps.

Volunteers. Three adults (age range, 25 to 30 years) participated as volunteers in this study after permission was obtained from our university's ethics committee. Volunteers were thoroughly briefed on the experimental protocol and the infectious agent to be used before their written consent was obtained.

Just before each experiment, the volunteers were required to wash their hands with warm tap water, rinse them with 70% ethanol, and allow them to air dry in order to minimize the influence of accumulated materials such as emollients on virus survival (2). Immediately after each experiment, the fingerpads were decontaminated by being pressed for 3 min on tissue paper soaked in a 6% solution of sodium hypochlorite (Javex Domestic Bleach; Colgate-Palmolive, Toronto, Ontario, Canada). The volunteers were then required to wash their hands thoroughly with liquid soap and warm tap water.

Virus recovery from disks. The virus was suspended in bovine mucin, bovine serum, or nasal discharge, and 10 μl of the test suspension was placed at the center of each stainless steel disk with a positive displacement pipette (Gilson Medical Instruments, Villiers-le-Bel, France). The disk was immediately picked up with sterile forceps and placed in a glass vial containing 990 μl of EBSS. The contents of the vial were sonicated in a bath (Bransonic Ultrasonic Corp., Danbury, Conn.) for 10 min, and the eluate was diluted and plaque assayed along with the input virus control.

Testing for virucidal activity. The disk carrier protocol used for this purpose has been described in detail before (26, 29, 39). Briefly, each disk was inoculated with 10 μl of the virus suspension under test. The inoculum was allowed to become visibly dry, which occurred in 30 to 70 min at room temperature (22 ± 2°C) depending on the level of relative humidity (RH). Each disk was then covered with 20 μl of the disinfectant under test; control disks received 20 μl of tap water (approximately 0.5 ppm of free chlorine) instead. At the end of the contact period, the disk was placed in a vial containing 980 μl of EBSS to arrest disinfectant action by dilution and to elute the virus; in experiments with the bleach, the eluent also contained 0.1% sodium thiosulfate (BDH) to ensure the complete and rapid neutralization of chlorine. The 1:50 initial dilution of all the disinfectants in the EBSS eluent was completely nontoxic to HeLa cells. Preexposure of the cell monolayers to it also did not interfere with the plaque formation of the rhinovirus. When 990 μl of a 1:50 dilution of the disinfectants in EBSS was mixed separately with 10 μl of the mucin-suspended virus and the mixtures were held for 1 h at room temperature, there was no detectable loss in virus infectivity, showing the absence of any residual virucidal activity in the eluent.

The eluates were diluted in EBSS and plaque assayed. In these experiments, additional controls were included to determine the level of infectious virus in the input inoculum placed on each disk (0-min control) and that surviving the drying process (dry control). Each product was tested at least three times, and in each test no fewer than three disks were treated with the product under test. We generally regard a product as effective if it can reduce the infectivity titer of the virus by at least 99.9%.

TABLE 2. Efficiency of recovery of rhinovirus type 14 from the carrier by using EBSS

Suspending medium and expt	Input PFU (10^5)	PFU recovered (10^5)	% Recovery
Serum, 5%			
1	1.45	1.30	90
2	1.40	1.80	129
3	4.00	1.60	40
4	4.00	4.70	118
Mean ± SD			94.3 ± 39.7
Mucin			
5	1.80	1.79	97
6	1.10	1.20	109
7	6.00	5.50	92
8	4.70	4.30	90
9	8.00	5.10	64
10	21.00	22.00	105
11	11.00	9.30	85
12	0.50	0.65	130
13	1.90	1.90	100
Mean ± SD			96.9 ± 18.1

Virus transfer experiments. The virus transfer protocol has been described in detail before (1a, 28). Virus transfer from disk to finger was tested with and without treating the dried virus inocula on the disks with the disinfectant under test. The dried inoculum on the disk (see above) was first covered with 20 μl of either the test product or tap water and allowed to dry under ambient conditions. For virus transfer, the disk was placed at the center of the pan of a Sartorius balance (model 1206 MP; Göttingen, Germany), and the demarcated area of the fingerpad was pressed on it at approximately 1 kg/cm^2 for 10 s; this was deemed to be equivalent to the pressure routinely applied in contact with environmental surfaces. After the contact, virus was eluted from the fingerpad in a vial with 1 ml of EBSS, as described in detail before (1a, 28), and the eluates were plaque assayed. Briefly, the contaminated area of the fingerpad was placed firmly over the mouth of the vial, and the vial was inverted with the fingerpad still over it and held in position for 5 s. This was followed by 20 full inversions of the vial with the fingerpad still over it and an additional 5 s of soaking and 20 more full inversions. The surface of the fingerpad was then scraped on the inside rim of the vial to recover as much fluid as possible. A recording hygrothermograph (Cole-Parmer Instrument Co., Chicago, Ill.) was used to continuously monitor the air temperature and RH of the atmosphere. Each experiment was repeated at least three times with a minimum of three disks for transfer in each.

Statistical analysis. A computerized statistical program, Statistical Analysis System, was used to analyze the data.

RESULTS

Efficiency of rhinovirus recovery from the disks. As can be seen from the data summarized in Table 2, the virus recov-

TABLE 3. Rhinovirus survival after the time required to dry the inoculum on the disks

Virus-suspending medium	No. of expts	% of initial titer recovered
5% serum	4	29.8 ± 12.0
Mucin	9	21.7 ± 6.9
Nasal discharge	3	15.2 ± 8.6

ery from the disks was 94.3% ± 39.7% and 96.9% ± 18.1% with the serum- and mucin-suspended virus, respectively, when the input virus per disk ranged from 0.5×10^5 to 2.1×10^6. In certain experiments, the number of virus PFU in the eluate was higher than that in the input inoculum. Since monodispersed virus preparations were not used in this study, the elution procedure may have resulted in the breakup of some clumps of infectious virus particles.

Rhinovirus survival after the initial drying of the inoculum on disks. The virus inoculum on the disks was allowed to become visibly dry under ambient conditions, and the RH level was monitored during the drying period. These experiments were conducted from the beginning of January to the end of August 1992, and on the days the inoculated disks were dried the RH ranged from 32 to 50%. As expected, there was generally a direct relationship between the RH level and the drying time for the inoculum, irrespective of the type of virus-suspending medium used. When the RH was at the lower level (32 to 35%), the drying time was between 29 to 33 min, and at the higher level of RH (45 to 50%) it was 60 to 90 min. As can be seen from Table 3, the amount of virus surviving when the inoculum became visibly dry was 21.7% ± 6.9%, 29.8% ± 12.0%, and 15.2% ± 8.6% for the mucin-, serum- and nasal discharge-suspended viruses, respectively. The differences in the values for virus survival in the three suspending media were not found to be statistically significant.

Loss in rhinovirus infectivity titer after disinfectant treatment. The virus inoculum dried on each disk was exposed to 20 μl of the disinfectant under test for a desired contact time, and the virus was eluted and plaque assayed. The results of these comparative tests are presented in Table 4.

LDS was able to reduce the plaque titer of the rhinovirus by >99.99% after a contact of 10 min irrespective of the type of virus-suspending medium used. Experiments for a contact period of 1 min were conducted with mucin-suspended virus only, and LDS proved to be equally effective at this reduced contact time as well. The remaining three products were tested against mucin-suspended virus only. In only one experiment the bleach at 800 ppm of free chlorine was able to produce a >99.9% reduction in the titer of the virus after a contact time of 10 min. When the bleach was tested at 500 ppm of free chlorine, it was able to reduce the virus titer by only 94% even after a contact time of 10 min. The quaternary ammonium compound and the phenolic were much less effective against the rhinovirus, reducing its infectivity titer by only 14.7 and 62.3%, respectively, after a contact time of 10 min. In one experiment with the quaternary, there was no detectable drop in the titer of the virus.

Rhinovirus transfer from disks to hands. Volunteers were asked to press a randomly selected fingerpad on either a dry control or a disinfectant-treated disk. Virus was immediately but separately eluted from the fingerpad as well as the disk and plaque assayed. In five separate experiments with LDS and two separate experiments with the bleach at 800 ppm of free chlorine, no infectious virus could be detected on any of the fingerpads after contact with the disinfectant-treated disks. Also, no infectious virus could be recovered from the disks after fingerpad contact. This is not unexpected in view of the high levels of rhinovirus inactivation on the disks by these two products. In contrast to this, 53.8% ± 15.8% and 21.2% ± 17.1%, respectively, of the virus surviving the initial drying of the inoculum remained detectable even after the disks were treated with the quaternary ammonium and the phenolic and allowed to dry. Furthermore, 8.4% ± 3.6% and 3.3% ± 1.9% of the infectious virus on the dried quaternary ammonium- and phenolic-treated disks, respectively, could be transferred to the fingerpads (Table 5).

Six separate experiments, using three volunteers, were conducted to test infectious virus transfer to fingerpads from disks in which the inoculum was allowed to become visibly dry. The drying time ranged from 30 to 39 min, and 0.58% ± 0.35% of the PFU surviving the drying process could be transferred (Table 5). Therefore, the amount of virus transferred after exposure of the disks to the quaternary ammonium compound and the phenolic was severalfold higher than that from the control disks. The values for virus transfer

TABLE 4. Loss in rhinovirus type 14 infectivity titer after treatment with tap water or disinfectant

Treatment and suspending medium	No. of expts	Contact time (min)	Virus PFU (10^4 [mean ± SD])		% Loss (mean ± SD)
			Before treatment	After treatment	
Tap water, mucin	3	10	2.30 ± 0.85	1.1 ± 0.47	52.2 ± 17.5
Lysol					
5% serum	3	10	5.28 ± 0.41	ND[a]	>99.99
Mucin	3	1	14.50 ± 3.50	ND	>99.99
Mucin	12	10	4.85 ± 3.40	ND	>99.99
Nasal discharge	1	10	1.4	ND	>99.99
Bleach (800 ppm)					
Mucin	3	10	14.60 ± 11.50	0.03 ± 0.40	99.70 ± 0.18
Mucin	1	1	17.00	0.02	99.84
Bleach (500 ppm)					
Mucin	1	10	1.50	0.09	94.10
Mucin	1	1	1.50	0.17	88.70
Quaternary, mucin	3	10	3.50 ± 1.60	3.00 ± 1.30	14.70 ± 14.50
Phenolic, mucin	3	10	3.00 ± 1.70	1.10 ± 0.59	62.3 ± 18.10

[a] ND, not detected.

TABLE 5. Transfer of mucin-suspended and dried rhinovirus type 14 from disinfectant-treated disks to fingerpads after a 10-s contact at a pressure of 1.0 kg/cm^2

Treatment and no. of expts	PFU (10^4 [mean ± SD])		% PFU (mean ± SD) transferred
	After drying of disinfectant	Transferred to fingerpad	
Untreated (dry control), 6		0.24 ± 0.20	0.58 ± 0.35
Lysol, 5	ND[a]	ND	0
Bleach (800 ppm), 2	ND	ND	0
Quaternary, 3	1.60 ± 0.40	0.13 ± 0.06	8.40 ± 3.60
Phenolic, 3	0.52 ± 0.60	0.015 ± 0.018	3.30 ± 1.9

[a] ND, not detected.

after treatment with the quaternary and the phenolic were both significantly different from the control ($P > 0.0034$).

DISCUSSION

It has been argued previously (40) that handwashing, although crucial to preventing transmission of infections, remains insufficient in the presence of a contaminated environment which can recontaminate recently washed hands. Demonstration of the utility of surface disinfection by epidemiological studies is extremely difficult and costly. We have therefore used a laboratory-based protocol for this purpose.

A rhinovirus was selected here because (i) these agents are responsible for a large proportion of the cases of the common cold in the general community (10) as well as in institutional settings (5, 11), (ii) the mechanisms of spread of rhinovirus colds are at least partially understood (19, 20), (iii) they are nonenveloped and therefore have a higher degree of resistance to many commonly used disinfectants (40), (iv) they are relatively safe for human volunteers, and (v) they are known to have a minimal infective dose of less than 1 cell culture infective unit from human volunteer studies (20).

The disinfectant products selected represent four major classes of environmental surface disinfectants. Bleach is one of the most commonly used disinfectants in a variety of settings; the use dilution chosen is based on the recommendation for the use of such chlorine-based products in day care centers (16) and health care settings (41). LDS is an alcohol-based spray sold for household and institutional use, whereas the quaternary ammonium compound and the phenolic tested are intended for industrial and institutional use only. The label for LDS states that the product is to be sprayed for 2 to 3 s on the surface to be decontaminated, and the treated surface should be allowed to air dry for at least 10 min. The use of the disk carrier protocol, however, made it necessary to first spray this product into a clean and sterile plastic tube and then apply the collected fluid (20 μl) to the dried inoculum on each disk. This slight change in the application of LDS probably presented the disinfectant with a higher degree of challenge than in the EPA protocol (42) and is not likely to have overestimated its rhinovirucidal activity.

LDS, the quaternary ammonium, and the phenolic make label claims for broad-spectrum germicidal activity, but only LDS specifically lists a rhinovirus among the viruses it can inactivate; testing of this product by our disk carrier protocol corroborates this claim. In fact, LDS could reduce the plaque titer of the rhinovirus by >99.9% in a contact time of 1 min. On the other hand, bleach at 800 ppm of free chlorine was only able to achieve 99.9% inactivation in one of three experiments utilizing a contact time of 10 min. The quaternary and the phenolic were unable to do so even after a contact time of more than 30 min. This further confirms our earlier observation (38) that if a product (except formaldehyde) could not inactivate rotaviruses on a hard surface in the first few seconds, it was unable to do so even when the contact time was extended. Here it is also interesting to note that the pHs of the use dilutions of both the quaternary and the phenolic tested in this study were found to be less than 3. In spite of this, they were unable to inactivate the rhinovirus. The loss is virus titer after tap water treatment was higher than that seen with the quaternary ammonium (Table 4). Whereas more work is needed to determine the reason(s) for this finding, one possible explanation for it is the neutralization of residual chlorine in the tap water by components of the quaternary ammonium product (9).

The lack of any detectable virus transfer from the LDS- and bleach-treated disks was somewhat predictable in view of the high level of their activities against the virus. The extent of virus transfer from the quaternary ammonium- and phenolic-treated disks was, however, unanticipated.

LDS is recommended for use on precleaned surfaces. The other three products are sold as single-step cleaner-disinfectants, and surfaces to be treated by them do not require precleaning because of the cleaning action in their formulation. Whereas eliminating the need to preclean a contaminated surface is considered a desirable attribute in a disinfectant, the findings of this study indicate that if a product is an ineffective germicide, its detergent action may help spread the contamination over a wider area when the treated surface is wiped. The fact that almost 10-fold-more virus was recovered from the fingerpads after contact with the phenolic- and quaternary-treated surfaces, relative to a nontreated surface, echoes this point. The potential for spread of contamination is particularly true of horizontal surfaces, where rinsing is difficult. Small objects which can be completely immersed and rinsed may be effectively treated by detergent-disinfectant combinations. However, care must be exercised in handling and disposal of wash water, since it may contain infectious virus.

Earlier studies on virus survival showed bovine mucin to be a suitable suspending medium for working with human rhinoviruses (37). In this investigation, its use as an organic load was also found to be appropriate, because the virucidal activity of LDS was the same when the test virus was suspended in the mucin, 5% fetal bovine serum, or nasal discharge; 5% fetal bovine serum is the organic load recommended in the EPA guidelines for virucidal tests (42).

Throughout this study, the drying of the virus inoculum on the disks was carried out strictly under ambient conditions. Even though this resulted in the differences in the drying time of the inoculum from experiment to experiment, this was considered to be more representative of the variations in nature. Incorporation of the proper controls compensated for the lack of manipulation of the room temperature and the RH. The disk protocol was adopted for this study on the basis of our prior studies with a number of other viruses. Unlike the EPA carrier test for virucides (42), the disk protocol lends itself readily for testing the virucidal activity of disinfectants as well as for conducting the transfer tests.

The virus elution procedure was able to recover nearly all the infectious virus placed on the disks when the recovery was tested immediately after the disks were inoculated. Was the elution just as efficient when the inoculum was allowed to dry or after it was treated with a disinfectant? This question

can be answered properly only by using virus particles with a suitable physical tracer incorporated into them. Whereas we did not conduct such experiments with the rhinovirus, our earlier studies with hepatitis A virus (27) and a human adenovirus (22) have shown that the elution procedure was just as effective in virus recovery even when the inoculum was dried and after it was treated with disinfectants such as LDS, diluted bleach (1,000 ppm of free chlorine), and 2% alkaline glutaraldehyde.

The elution procedure also permitted a simultaneous 50-fold dilution of the disinfectant in the eluent. This level of dilution was sufficient to immediately arrest the virucidal activity of the product under test and to render it nontoxic to the cell cultures. The residue of the disinfectant in the eluent was also unable to interfere with the plaque-forming ability of the rhinovirus. The amount of sodium thiosulfate in the eluent for the chlorine experiments was also harmless to the cells and noninterfering with the plaque assay.

Even though the volume of the inoculum placed on each disk was relatively small, the amount of infectious virus in it was higher than what might be expected in a unit volume of nasal discharge from a case of rhinovirus cold. The use of a higher level of virus was essential to determine the minimum 3.0 $\log_{10}$ unit reduction in virus plaque titer.

The contact time and pressure used in the transfer experiments are considered reasonably representative of some routine interaction between hands and fomites or environmental surfaces. The results of virus transfer (Table 5) from disks with dried but untreated inocula agree well with those of our earlier studies with rhinovirus type 14 (3). Whereas application of friction during the contact could transfer a larger amount of the virus, friction was not applied when transfer was attempted from disinfectant-treated disks to keep the number of experiments limited. If it is assumed that the average level of infectious virus in nasal secretions is 100-fold less than the amount placed on the disks, the number of infectious units transferred would still be several times higher than its minimal infective dose.

In this study, only the intact surface of the fingerpads was exposed to cell culture-grown virus, thereby minimizing the health risk to the volunteers. The procedure used for eluting infectious virus from the fingerpads has already been shown to be very efficient and reproducible (1a, 28). The use of fingerpads rather than whole hands also provided more statistical efficiency by allowing replicates and comparisons in the same experiment.

Nonenveloped viruses are relatively resistant to inactivation by the levels of quaternary ammonium compounds and phenolics used in formulations for the disinfection of environmental surfaces (40). Therefore, the activity of LDS against the rhinovirus is most likely due to the ethanol rather than the small amount of *o*-phenylphenol in its formulation. Recently, Ward et al. (44) showed that LDS was also effective in interrupting the transfer of rotaviruses from environmental surfaces to susceptible human volunteers.

A study carried out nearly 10 years ago (12) showed LDS to be moderately effective in reducing the spread of rhinoviral infections through the disinfection of fomites and environmental surfaces. The protocol used was semiquantitative, and the formulation of the product used then is different from the one on the market now. A more recent study (4) using the current formulation of LDS also found the product to be among the more effective of the 39 products tested; the virucidal tests with poliovirus type 1 were, however, conducted with a suspension test.

The findings of this study clearly show that certain disinfectants can interrupt the transfer of rhinoviruses from environmental surfaces to hands. However, the selection of a given product for routine use must be based on its safety for human health and the environment, compatibility with the surfaces to be treated, ease of storage and application, and the spectrum of germicidal activity. The methods and information generated in this study should make it possible to test the usefulness of environmental surface disinfection in preventing the transmission of rhinovirus colds in settings such as those designed by Meschievitz et al. (30). Any such studies in the future should also address the crucial questions of what environmental surfaces should be disinfected and how often in order to successfully reduce the spread of rhinoviruses and other similar infectious agents, especially in institutional settings.

Rhinoviruses continue to be a significant burden on the health care system and the economy in general (43). Apart from the cases they cause in the community (11), rhinoviruses frequently give rise to outbreaks in institutions (7, 13, 24, 25), such as schools, day care centers, hospitals, etc. Therefore, any reasonable approach that can help reduce the spread of these infections should receive due consideration.

Recent advances in our understanding of the structure and biology of rhinoviruses (34) have had little impact so far in reducing the health and economic burden (43) due to rhinoviral infections. It is, therefore, important that alternate means of preventing and controlling the spread of these viruses be explored while attempts at the development of vaccines and drugs against them are also under way. The focus of this study was one such alternative, and its findings indicate that proper disinfection of environmental surfaces can effectively interrupt rhinovirus spread to hands.

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