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Chemical Disinfection of Hepatitis A Virus on Environmental Surfaces

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Hepatitis A virus disinfection was assessed on contaminated stainless-steel disks. Ten microliters of fecally suspended hepatitis A virus was deposited on the center of each disk, dried for 20 min, and then covered with 20 μl of the test product for 1 min. Of the 20 formulations tested, only 2% glutaraldehyde, a quaternary ammonium formulation containing 23% HCl (toilet bowl cleaner), and sodium hypochlorite (>5,000 ppm [>5,000 μg/ml] of free chlorine) reduced the virus titer by >99.9%; phenolics, iodine-based products, alcohols, and solutions of acetic, peracetic, citric, and phosphoric acids were unable to do so.

Outbreaks of hepatitis A are frequently associated with eating establishments (11), hospitals (8), day-care centers (6), and schools (12). Chemical disinfection of environmental surfaces and hand antiseptics are relied upon to prevent and control such outbreaks, but not enough is known about the efficacy of such products in the inactivation of hepatitis A virus (HAV). The present study concentrates on the efficacy of commonly used surface disinfectants under simulated field conditions.

A seed culture of FRhK-4 cells was kindly provided to us by M. D. Sobsey, University of North Carolina, Chapel Hill. The cultivation and maintenance of the cells were as described by Sobsey et al. (17). Briefly, Eagle minimum essential medium (GIBCO, Grand Island, N.Y.) with 5% fetal calf serum, 50 μg of gentamicin sulfate (Cidomycin; Roussel, Montreal, Quebec, Canada) per ml, 50 μg of kanamycin (GIBCO) per ml, 0.015 M HEPES (*N*-2-hydroxyethyl piperazine-*N*-2-ethanesulfonic acid; GIBCO), and 0.113% sodium bicarbonate (BDH Chemicals, Toronto, Ontario, Canada) was used for growing the cells. The cells were maintained in the same medium but containing only 2% fetal calf serum.

The HM-175 strain of HAV was also received from M. D. Sobsey. Stock virus was prepared by infecting FRhK-4 monolayers at a multiplicity of infection of 0.01. The virus was allowed to adsorb for 90 min at 37°C, and the infected cultures were then kept in the maintenance medium until 75% of each monolayer was affected by virus cytopathology. The cultures were frozen (−20°C) and thawed three times, and the culture fluid was clarified by centrifugation for 10 min at 1,000 × *g*. The virus was concentrated by polyethylene glycol hydroextraction as described by Ramia and Sattar (13). Dialysis tubing (Spectrum Medical Industries Inc., Los Angeles, Calif.) containing 100 ml of the clarified cell culture fluid was placed in a plastic tray and completely covered with polyethylene glycol (molecular weight, 8,000; Matheson, Coleman and Bell, Norwood, Ohio). After overnight hydroextraction at 4°C, the residue in the dialysis tube was suspended in 2 ml of Earle balanced salt solution. The virus concentrate was divided into aliquots for storage at −70°C.

Plaque assays of the virus were carried out in FRhK-4 monolayers in 12-well plastic plates (Costar, Cambridge, Mass.). Three wells were used for each virus dilution tested. Each well received 0.1 ml of the inoculum, and the virus was allowed to adsorb for 90 min at 37°C. The overlay consisted of Eagle minimum essential medium, 2% fetal calf serum, 50 μg of gentamicin sulfate per ml, 50 μg of kanamycin sulfate per ml, 2 μg of amphotericin B (Fungizone) per ml, 0.015 M HEPES, 0.113% sodium bicarbonate, 0.75% agarose (type II; Sigma Chemical Co., St. Louis, Mo.), and 26 mM magnesium chloride (BDH). The plates were sealed in laminated plastic bags (Philips, Toronto, Ontario, Canada) and incubated for 8 days at 37°C. The procedure for fixing and staining the monolayers prior to counting plaques has been described before (14).

Stainless-steel disks (1 cm in diameter), punched out of locally purchased no. 4 finish polished sheets, were used as carriers to represent nonporous inanimate environmental surfaces. The method for their decontamination, cleaning, and sterilization before reuse has been described in detail previously (14).

The selection of the disinfectant formulations tested in this study was based on (i) manufacturer-recommended use for surface disinfection, (ii) type(s) and level(s) of active ingredients, and (iii) results of previous tests with other viruses (10, 14). Unless otherwise stated, tap water was used for diluting a given product according to the directions on the label. Free chlorine in the chlorine-based disinfectants was estimated by the DPD (*N*,*N*-diethyl-*p*-phenylenediamine) method, using a commercial kit (Hach Chemical Co., Ames, Iowa).

The fecal sample used in this study was obtained from a healthy 5-month-old baby. It was prepared as a 10% suspension in normal saline, clarified of gross particulate matter by 10-min centrifugation at 1,000 × *g*, and passed through a 0.2-μm membrane filter (Nalge Co., Rochester, N.Y.) to remove bacteria and fungi. The filtrate was found to be free of cytotoxicity and indigenous viruses when tested by the procedure described before (10) and therefore was used as an organic load in this study.

A positive-displacement pipette (Gilson Medical Instruments, Villiers-le-Bel, France) was used to deposit 10 μl of the fecally suspended HAV at the center of each disk placed in the wells of 24-well plastic cell culture plates (Corning). The inoculum was allowed to dry by keeping the disks for 20 min in a laminar flow hood. Twenty microliter of the product under test was then placed over the dried inoculum and

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allowed to remain in contact for 1 min under ambient conditions. At the end of the contact time, each disk was placed in a glass vial containing 980 μl of tryptose phosphate broth (Difco, Detroit, Mich.). The contents of the vial were sonicated (Bransonic Ultrasonic Corp., Danbury, Conn.) for 10 min to achieve maximal elution of the virus from the disks. Control disks were treated in an identical manner except that, instead of a disinfectant, they received 20 μl of Earle balanced salt solution after 20 min of drying of the inocula. In each experiment at least three disks were treated with the formulation under test, and each experiment was repeated at least once. Therefore, the results given in Table

TABLE 1. Efficacy of chemical disinfectants against HAV in a carrier test

Disinfectant[a]	Dilution tested	pH	Recommended for:	Log reduction
Commercial formulations				
(1) 2.0% glutaraldehyde	Undiluted	7.5	Medical instruments & appliances	>4
(2) 6% sodium hypochlorite	1:18 (3,000 ppm free chlorine)	10.9	General purpose disinfection	<1
	1:11 (5,000 ppm free chlorine)	11.2	General purpose disinfection	>4
(3) 2.73% sodium chlorite & 15.10% organic acid	1:12	2.9	Medical instruments & environmental surfaces	<1
(4) 15.5% iodophor no. 1 & 6.5% phosphoric acid	1:214 (75 ppm titratable iodine)	2.8	Highly contaminated areas, isolation rooms & food contact surfaces	<1
(5) 9.1% iodophor no. 2 & 8.74% iodophor no. 3	1:173 (75 ppm titratable iodine)	5.2	Surfaces & skin	<1
(6) 0.5% sodium *o*-benzyl-*p*-chlorophenol & 0.6% sodium lauryl sulfate	Undiluted	12.7	Environmental surfaces	<1
(7) 0.1% *o*-phenyl-phenol, 70% ethyl alcohol, & 0.14% chlorhexidine gluconate	Undiluted	2.4	Surfaces & instruments in medical, dental, & veterinary clinics	<1
(8) 0.4% QAC no. 1 and 23% HCl	Undiluted	0.4	Toilet bowls, urinals, and enamel surfaces	>4
(9) 10.76% QAC no. 2, 10.76% QAC no. 3, & 16% isopropanol	1:500	6.3	General purpose surface & equipment cleaning	<1
(10) 0.3% QAC no. 4, 45% isopropyl alcohol, 3% triethylene glycol, & 2% propylene glycol	Undiluted	7.6	Environmental surfaces	<1
(11) 2.7% QAC no. 5	Undiluted	9.2	Environmental surfaces	<1
	1:33	8.9	Environmental surfaces	<1
(12) 2.7% QAC 5 & 70% ethanol	Undiluted	10.1	Environmental surfaces	<1
Noncommercial formulations				
(13) 10% chloramine-T	1:18 (3,000 ppm free chlorine)	7.7	Food equipment & environmental surfaces	<1
	1:11 (5,000 ppm free chlorine)	8.0	Food equipment & environmental surfaces	<1
(14) 3.125% iodophor no. 4	Undiluted	1.8	Instruments & environmental surfaces	<1
(15) 70% ethanol	Undiluted	5.2	Surface disinfection	<1
(16) 99.8% acetic acid	1:33	2.6	Environmental surfaces	<1
	1:10	2.2	Environmental surfaces	<1
(17) 35% peracetic acid	1:33	2.4	Isolators, medical & surgical equipment, & environmental surfaces	<1
	1:10	2.2	Isolators, medical & surgical equipment, & environmental surfaces	<1
(18) 85% phosphoric acid	1:33	2.3	Environmental surfaces	<1
	1:10	1.8	Environmental surfaces	<1
(19) 10% citric acid	1:3	2.9	Environmental surfaces	<1
	1:2	2.6	Environmental surfaces	<1
(20) 30% hydrogen peroxide	1:10	6.8	Surgical implants, ventilators, utensils, & thermoplastic equipment	<1
	1:5	6.6	Surgical implants, ventilators, utensils, & thermoplastic equipment	<1

[a] QAC no. 1, *n*-Alkyl (50% C-14, 40% C-12, 10% C-16) dimethyl benzyl ammonium chloride; QAC no. 2, *n*-alkyl (60% C-14, 30% C-16, 5% C-12, 5% C-18) dimethyl benzyl ammonium chloride; QAC no. 3, *n*-alkyl (68% C-12, 32% C-14) dimethyl ethylbenzyl ammonium chloride; QAC no. 4, *n*-alkyl (40% C-12, 50% C-14, 10% C-16) dimethyl benzyl ammonium chloride; QAC no. 5, *n*-alkyl (50% C-14, 40% C-12, 10% C-16) *n*-*n*-dimethyl *n*-benzyl ammonium chloride; iodophor no. 1, butoxypolypropoxypolyethoxy ethanol-iodine complex; iodophor no. 2, polyethoxypolypropoxypolyethoxy ethanol-iodine complex; iodophor no. 3, nonylphenoxypoly(ethyleneoxy) ethanol-iodine complex; iodine no. 4, trial formulation.

1 are based on the means and standard deviations from a minimum of six replicate observations. A formulation was regarded as effective against HAV when it could reduce the virus infectivity titer by at least 99.9% under the test conditions described.

Table 1 lists the 20 disinfectant formulations tested in this study and the results obtained with each. Freshly activated 2% glutaraldehyde solution reduced the HAV titer to an undetectable level in our system. This is consistent with results obtained for other viruses on contaminated surfaces (10, 14) and suggests that 2% alkaline glutaraldehyde is effective against HAV even in the presence of an organic load. Glutaraldehyde is highly recommended for the disinfection of medical instruments and appliances such as endoscopes which cannot be heat sterilized (18), a recommendation supported by our results on its activity against HAV. It is not known whether glutaraldehyde concentrations of <2% would be active against HAV. Recent studies with mycobacteria (5) and other enteroviruses (Sattar et al., unpublished data) have shown that alkaline glutaraldehyde may be ineffective against these agents when tested at concentrations below 2%. There is also a need to test the efficacy of glutaraldehyde against HAV under conditions in which the disinfectant is reused.

Our results suggest that sodium hypochlorite is more effective than chloramine-T against HAV; at 5,000 ppm (5,000 μg/ml) free chlorine, chloramine-T had little effect on virus titer, whereas at the same concentration of free chlorine, sodium hypochlorite decreased virus titer to undetectable levels. Sodium hypochlorite is a broad-spectrum, inexpensive, and fast-acting disinfectant; it is, however, known to be extremely sensitive to the presence of organic material. Sattar et al. (14) also found that at least 5,000 ppm of available chlorine was required for the disinfection of coxsackievirus B3 under similar test conditions. Similarly, inactivation of other non-enveloped enteric viruses required high levels of free chlorine (10, 14). The recommended disinfection concentration for chlorine is 500 to 5,000 ppm (4). The results of our studies suggest that free chlorine concentrations in excess of 5,000 ppm be used for effective disinfection of surfaces contaminated with HAV and other nonenveloped viruses.

Three iodine-based compounds were tested for their efficiency to disinfect HAV on environmental surfaces. None of them was effective against HAV. Other studies have similarly found virucidal efficacy of iodophors to be limited (3, 10, 14).

Quaternary ammonium compounds (QAC) are widely used as disinfectants. In this study, five QAC-based formulations were examined and only one proved to be effective against HAV. This effective formulation contained 23% HCl and had a pH of 0.42, suggesting that its activity was due to the acid and not the QAC itself, since other QAC with a higher concentration and/or in combination with other compounds such as alcohols were inactive against HAV. Ethanol or isopropanol alone, or isopropanol with triethylene and propylene glycol, when added to QAC did not improve the activity of QAC. The results obtained in this and previous studies (10, 14) suggest that QAC are unreliable virucidal disinfectants. Phenolic compounds tested in this study were unsatisfactory as HAV disinfectants. One of the phenol compounds contained ethanol and chlorhexidine gluconate, but had no better HAV-inactivating capacity than the other, which contained chlorophenate and sodium lauryl sulfate.

Isopropyl alcohol in combination with chlorhexidine gluconate has been reported to be effective against rotaviruses (10). In chimpanzee transmission experiments (3), 70% isopropyl alcohol and 80% ethanol are reported effective against hepatitis B virus within 10 and two min, respectively. This was not the case with HAV; alcoholic disinfectants on their own, or in combination with QAC, ethylene, and propylene glycol, with chlorhexidine gluconate and phenol, or with iodine and phosphoric acid were insufficient to inactivate this virus within 1 min contact time.

All four acids tested were unable to inactivate HAV on environmental surfaces even when the pH level for some of them was as low as 1.8. Only the acid containing QAC (pH <1.0), mentioned above, was effective. Enteroviruses as a group can remain viable at pH 3.0 (16), but HAV is even more acid resistant; recent studies by Scholz et al. (15) have shown that purified HAV can survive exposure to pH 1.0 for several minutes. The capacity of HAV to bind cellular material to its surface (9) may also play a role in protecting virus infectivity.

In the carrier test, peracetic acid was found efficacious against rotaviruses even in concentrations as low as 0.1% pure acid (10). In this study, peracetic acid concentrations of up to 3.5% (wt/vol) were unable to inactivate HAV infectivity. At both concentrations (3 and 6%), hydrogen peroxide was found ineffective against HAV on surfaces.

Institutional outbreaks of HAV are commonly reported, and in day-care centers, pediatric wards, eating establishments, and other institutions in general the infection may give rise to secondary cases in the general community (7). Recent studies have shown that HAV can remain infectious on nonporous inanimate surfaces for several days (Mbithi et al., unpublished data). Virus-contaminated surfaces can readily contaminate hands upon contact (1). Effective disinfection of HAV on environmental surfaces is therefore needed to facilitate the control of hepatitis A in institutions.

Before this study, information on efficiency of chemical disinfectants for HAV has been missing. The data presented indicate that the majority of chemical disinfectants now in use in both institutional and domestic environments do not inactivate HAV and cannot be considered as effective control measures. Only 2% glutaraldehyde, QAC with 23% HCl, and sodium hypochlorite with free chlorine in excess of 5,000 ppm can be recommended as effective surface disinfectants. Antiseptic efficacy, using an in vivo protocol (2), is being investigated in our laboratory.

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LITERATURE CITED

1. **Ansari, A. S., S. A. Sattar, V. S. Springthorpe, G. A. Wells, and W. Tostowaryk.** 1988. Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. J. Clin. Microbiol. **26**:1513–1518.
2. **Ansari, S. A., S. A. Sattar, V. S. Springthorpe, G. A. Wells, and W. Tostowaryk.** 1989. In vivo protocol for testing efficacy of hand-washing agents against viruses and bacteria: experiments with rotavirus and *Escherichia coli*. Appl. Environ. Microbiol. **55**:3113–3118.
3. **Bond, W. W., M. S. Favero, N. J. Peterson, and J. W. Ebert.** 1983. Inactivation of hepatitis B virus by intermediate-to-high-level disinfectant chemicals. J. Am. Med. Assoc. **18**:535–538.
4. **Centers for Disease Control.** 1982. Acquired immune deficiency syndrome (AIDS): precautions for clinical and laboratory staffs. Morbid. Mortal. Weekly Rep. **31**:557–580.
5. **Cole, E. C., W. A. Rutala, L. Nessen, N. S. Wannamaker, and D. J. Weber.** 1990. Effect of methodology, dilution, and expo-

sure time on the tuberculocidal activity of glutaraldehyde-based disinfectants. Appl. Environ. Microbiol. **56**:1813–1817.

6. **Hadler, S. C., and L. McFarland.** 1986. Hepatitis in daycare centers: epidemiology and prevention. Rev. Infect. Dis. **8**:548–557.
7. **Hadler, S. C., H. M. Webster, J. J. Erben, J. E. Swanson, and J. E. Maynard.** 1980. Hepatitis A in daycare centers—a community-wide assessment. N. Engl. J. Med. **302**:1222–1227.
8. **Klein, B. S., J. A. Michaels, M. W. Rytel, K. G. Berg, and J. P. Davis.** 1984. Nosocomial hepatitis A: a multinursery outbreak in Wisconsin. J. Am. Med. Assoc. **252**:2716–2721.
9. **Lemon, S. M., and L. N. Binn.** 1985. Incomplete neutralization of hepatitis A virus in vitro due to lipid-associated virions. J. Gen. Virol. **66**:2501–2505.
10. **Lloyd-Evans, N., V. S. Springthorpe, and S. A. Sattar.** 1986. Chemical disinfection of human rotavirus-contaminated inanimate surfaces. J. Hyg. **97**:163–173.
11. **Lowry, P. W., R. Levine, D. F. Stroup, R. A. Gunn, M. H. Wilder, and C. Konigsberg.** 1989. Hepatitis A outbreak on a floating restaurant in Florida, 1986. Am. J. Epidemiol. **129**:155–164.
12. **Naus, M., W. Everett, S. Davies, and J. Coutts.** 1989. A school outbreak of hepatitis A in South Western Ontario. Can. Dis. Weekly Rep. **15**:225–228.
13. **Ramia, S., and S. A. Sattar.** 1979. Second-step concentration of viruses in drinking and surface waters using polyethylene glycol hydroextraction. Can. J. Microbiol. **25**:587–592.
14. **Sattar, S. A., V. S. Springthorpe, Y. Karim, and P. Loro.** 1989. Chemical disinfection of non-porous surfaces experimentally contaminated with four human pathogenic viruses. Epidemiol. Infect. **102**:493–505.
15. **Scholz, E., U. Heinricy, and B. Flehmig.** 1989. Acid stability of hepatitis A virus. J. Gen. Virol. **70**:2481–2485.
16. **Siegl, G., M. Weitz, and G. Kronauer.** 1984. Stability of hepatitis A virus. Intervirology **22**:218–226.
17. **Sobsey, M. D., S. E. Oglesbee, and D. A. Wait.** 1985. Evaluation of methods for concentrating hepatitis A virus from drinking water. Appl. Environ. Microbiol. **50**:1457–1463.
18. **Van, A., M. Leriers, E. Serruys, and M. Cremer.** 1989. Methods of disinfecting endoscopic material: results of an international survey. Endoscopy **21**:247–250.