

THE EFFECT OF BLOOD ON THE ANTIVIRAL ACTIVITY OF SODIUM HYPOCHLORITE, A PHENOLIC, AND A QUATERNARY AMMONIUM COMPOUND

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ABSTRACT

OBJECTIVE: To assess the virucidal activity of three disinfectants (sodium hypochlorite, a phenolic, and a quaternary ammonium compound) in the presence and absence of blood.

METHODS: Disinfectants at varying concentrations (hypochlorite: 5,000, 500, or 50 ppm; phenolic: 1:10 or 1:128 dilution; quaternary ammonium compound: 1:10 or 1:128 dilution) were added to either saline or whole blood (final concentration, 80% or 20% blood) and mixed. Test organisms included an attenuated vaccine strain of poliovirus type 1 (prototype for relatively resistant hydrophilic viruses) and herpes simplex virus (HSV) type 1 (prototype for relatively susceptible lipophilic viruses). Virus was added to create a viral-blood suspension. Viral survival was tested at room temperature at the following times: 0, 15 seconds, 30 seconds, 1 minute, 2 minutes, 5 minutes, and 10 minutes. A neutralizer stopped the reaction, and virus was assayed using a plaque technique.

RESULTS: In the absence of blood, complete inactivation of HSV was achieved within 30 seconds with 5,000 (1:10 dilution of bleach) and 500 (1:100 dilution of bleach) ppm chlorine, 1:10 and 1:128 diluted phenolic (use dilution), and 1:10 and 1:128 diluted

quaternary ammonium compound (use dilution). In the presence of 80% blood, only 5,000 ppm hypochlorite, 1:10 phenolic, and 1:10 or 1:128 quaternary ammonium compound were effective. In the absence of blood, complete inactivation of polio was achieved within 30 seconds by 5,000 and 500 ppm chlorine and 1:10 quaternary ammonium compound. In the presence of 80% blood, no solution tested was capable of completely inactivating poliovirus within 10 minutes.

CONCLUSIONS: Our data suggest that, in the absence of visible blood, environmental surfaces may be disinfected with a diluted hypochlorite solution (1:10 or 1:100), a phenolic, or a quaternary ammonium compound. Based on our studies using HSV, which has similar susceptibilities to disinfectants as human immunodeficiency virus (HIV), phenolics at their use dilution and 1:100 diluted hypochlorite are unlikely to inactivate HIV or hepatitis B virus reliably in the presence of blood. Hypochlorite at a final concentration of 5,000 ppm (1:10 dilution) should be used to decontaminate blood spills, but, even after decontamination, care should be used to avoid sharps injuries (*Infect Control Hosp Epidemiol* 1999;20:821-827).

More than 20 infectious diseases have been transmitted via percutaneous injury with a contaminated needle or sharp.¹ Bloodborne pathogens of special importance include human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). The risks of transmission following a percutaneous injury with a sharp contaminated by one of these viruses have been reported as follows: HIV, approximately 0.30%; HBV, 6% to 30%³⁻⁵; and HCV, approximately 1.8%.⁶ Mucocutaneous or conjunctival exposure to blood also has led to acquisition of HIV^{2,7} and HCV.^{7,8}

Because of concerns regarding the transmission of bloodborne pathogens in the cleaning of blood spills, the Occupational Safety and Health Administration (OSHA) requires that blood spills be decontaminated with an appropriate disinfectant.⁹ The Centers for Disease Control and Prevention (CDC) recommends that blood spills be

promptly cleaned using an Environmental Protection Agency-approved germicide or a 1:100 solution of household bleach.¹⁰ Chlorine, phenolics, and quaternary ammonium compounds may be used to disinfect environmental surfaces contaminated with blood.¹¹ Concern has been raised that the virucidal efficacy of these disinfectants may be reduced in the presence of blood.

The purpose of this investigation was to determine the virucidal activity of chlorine, a phenolic, and a quaternary ammonium compound in the presence and absence of blood. Disinfectants at varying concentrations were tested for activity against a characteristic hydrophilic virus, poliovirus type 1, and against a lipophilic virus, herpes simplex virus type 1 (HSV-1). Hydrophilic viruses are generally more resistant to disinfectants than lipophilic viruses such as HSV-1, HIV, and HBV.¹²

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This study was supported in part by the Statewide Program for Infection Control and Epidemiology. The authors wish to thank Newman C. Aguiar, Douglas A. Wait, and Dorothy L. Thompson for their technical assistance. Dr. Steven Bachenheimer kindly provided the herpes simplex virus and VERO cell line and helpful advice. UNC animal surgery provided the blood.

99-OA-028. Weber DJ, Barbee SL, Sobsey MD, Rutala WA. The effect of blood on the antiviral activity of sodium hypochlorite, a phenolic, and a quaternary ammonium compound. Infect Control Hosp Epidemiol 1999;20:821-827.

METHODS

Viral Strains and Cell Culture

Poliovirus type I (strain LSc), a nonenveloped, single-stranded RNA virus, was produced in Buffalo green monkey kidney (BGMK) cells and isolated by Freon extraction. Cell lysates containing 10^8 plaque-forming units (PFU)/mL virus were stored at -70°C . Monolayers of BGMK cells were grown and maintained in Eagle's Minimum Essential medium (Sigma Chemicals, St Louis, MO) supplemented with 15 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (Gibco, Gaithersburg, MD), 2 mmol/L l-glutamine (Gibco), and 10% fetal calf serum (Sigma Chemicals) and antibiotics (50 $\mu\text{g}/\text{mL}$ gentamicin sulfate, 250 $\mu\text{g}/\text{mL}$ kanamycin sulfate, 150 $\mu\text{g}/\text{mL}$ mycostatin; Sigma) at 37°C in 5% CO_2 . Poliovirus infectivity was enumerated by the plaque technique in BGMK monolayers in 60-mm dishes (Fisher Scientific, Pittsburgh, PA). Virus titers were expressed as PFU.

HSV-1, an enveloped DNA virus, was kindly supplied by Dr. Steven Bachenheimer (University of North Carolina, Chapel Hill, NC). Viral stocks were propagated in VERO cells, an African green monkey kidney-derived cell line. Viral suspensions containing 10^9 PFU/mL were stored at -70°C . HSV-1 viral concentrations were determined using a plaque assay technique in VERO monolayers in 12-well plastic tissue culture plates (Corning Plastics, Corning, NY). Monolayers of VERO cells were grown in Dulbecco Modified Eagle's medium (Sigma Chemicals) with 4,500 $\mu\text{g}/\text{mL}$ glucose, 110 mg sodium pyruvate, antibiotics (see above), and supplemented with 5% fetal calf serum at 37°C in 5% CO_2 .

Disinfectant Solutions and Neutralizers

Three products were selected for evaluation: Clorox bleach (The Clorox Co, Oakland, CA), TBQ (Calgon-Vestal, St Louis, MO), and Vesphene Iise (Calgon-Vestal). Products tested, their active ingredients, and tested concentrations were as follows: sodium hypochlorite bleach, 5.25% sodium hypochlorite diluted to 5,000 ppm (1:10 dilution), 500 ppm (1:100 dilution), and 50 ppm (1:1,000 dilution); TBQ, a quaternary ammonium compound, 8% alkyl (50% C_{14} , 40% C_{12} , 10% C_{16}) dimethyl-benzyl-ammonium chlorides, at 1:10 and 1:128 dilutions (recommended use dilution, 1:128); and Vesphene Iise, a phenolic compound, 8.34% sodium p-tertiary-amylphenate and 9.65% sodium o-phenylphenate, at 1:10 and 1:128 dilutions (recommended use dilution, 1:128).

The products were stored in the dark at room temperature and tested within their specified use-life. Just prior to use, product dilutions were prepared using sterile distilled water as the diluent. Verification of product concentration was performed for sodium hypochlorite, and the concentration was found to be 5.2%, and a 1:10 dilution was completely neutralized by 1.1% sodium thiosulfate. Verification of other product concentrations was not performed.

For neutralization of Vesphene Iise and TBQ, a 1:10 dilution of test sample was made into Lethen Broth (Difco,

Detroit, MI) and Neutralization Broth (Difco), respectively. For sodium hypochlorite neutralization, a 1:10 dilution in 1.1% sodium thiosulfate was used.

Cytotoxicity of neutralized disinfectants in the absence of blood was determined by exposing BGMK and VERO cell monolayers to 0.1-mL volumes of dilutions 10^1 through 10^4 . Evidence for cytotoxicity was observed for Vesphene and TBQ, but the effect was eliminated at dilutions greater than 10^2 . Neutralizers were not antiviral or cytotoxic in either cell line nor did they influence the infectivity of test viruses. Additional tests indicated that saline and citric acid did not adversely affect viral infectivity.

Blood

All experiments using blood were conducted using fresh canine blood (University of North Carolina Animal Surgery, Chapel Hill, NC, and Lampire Biological Co, Pipersville, PA) that was stored at 4°C and used within its specified shelf life. Citric acid was included as an anticoagulant.

Titration of Available Chlorine

Total and free chlorine were measured in the presence and absence of blood. Concentrations of chlorine were measured by the ferrous titration method.¹³ Initial concentration of chlorine was 5.2%, but, after 10 minutes' exposure in 80% blood, no free chlorine could be measured. Total chlorine was determined to be 1,030 mg/L (ppm).

Determination of Antiviral Activity of Test Products Using a Suspension Test

Susceptibility testing of disinfectants was performed using a quantitative suspension test. The procedure was as follows: whole blood (final vol/vol 80%) was dispersed into a sterile glass tube, 1/10 test volume (eg, 0.7 mL-7.0 mL) of virus (7×10^7 PFU) was added, and the solution was mixed and then placed into a 20°C water bath. At the beginning of the timed interval, 1/10 final volume of test disinfectant was added to the blood-virus suspension. This suspension was maintained at 20°C , with samples removed at 15 seconds, 30 seconds, 1 minute, 2 minutes, 5 minutes, and 10 minutes. The removed samples were immediately diluted 1:10 with neutralization solution. Neutralized samples then were serially diluted in phosphate-buffered saline (PPBS) polio) or serum-free cell-culture medium (HSV-1) and placed in wet ice.

For the poliovirus plaque assay, BGMK cells were seeded into 60-mm Petri dishes and incubated for 3 days at 37°C in 5% CO_2 . Serial 10-fold dilutions of viral samples were assayed in triplicate for infectivity by inoculating confluent cell monolayers with 0.1 mL of viral dilutions. The virus was allowed to adsorb for 60 minutes at 37°C ; plates were tilted every 15 minutes to redistribute virus. After adsorption, 5 mL of 0.75% Eagle's Minimum Essential medium overlay medium containing neutral red was added. The cultures were held at 37°C for 48 hours, and monolayers were observed for evidence of viral cytopathology based on the presence of plaques.

For evaluation of HSV-1 samples, subconfluent 24-hour VERO cells seeded in 12-well tissue culture dishes were incubated with 0.1 mL of sample dilutions in triplicate. Inoculated monolayers were incubated for 90 minutes; plates were tilted every 15 minutes to redistribute virus. After adsorption, 2 mL of medium containing 0.5% agarose (Type I, Sigma) was added to each well. Plates were incubated at 37°C in 5% CO₂ for 2 to 3 days. The cell monolayer was stained with 0.5% crystal violet for 5 minutes, then rinsed in distilled water. Plates were allowed to air dry at room temperature prior to plaque enumeration.

Control samples consisted of untreated virus suspensions serially diluted in PBS or serum-free medium and whole blood (80% and 20%). Negative controls were diluted in medium and neutralizer alone. For each experimental trial, untreated viral controls were included with treated samples. All assays of control dilutions, as with treated samples, were performed in triplicate.

Two-Dose Trials

For the two-dose trials, the above procedures were followed, but an additional 0.5 mL of 5,000 ppm (1:10 dilution) of chlorine was added to the viral suspension at 45 seconds and the suspension vortexed. After the addition of the second dose of hypochlorite, samples were removed at 1, 2, 5, and 10 minutes (timed from the initiation of the experiment) and added to 9.0 mL of neutralizer. Tenfold serial dilutions in PBS were prepared and evaluated as described above. Due to the addition of the second dose of hypochlorite, the final blood concentration was 73%.

RESULTS

Efficacy of Disinfectants Against Poliovirus in the Absence of Blood

Three concentrations of hypochlorite were tested for efficacy against poliovirus: 5,000 ppm (1:10 dilution), 500 ppm (1:100 dilution), and 50 ppm (1:1,000 dilution; Table 1). In the absence of blood, four trials using 5,000 ppm and one trial using 500 ppm revealed that viral inactivation was beyond the limit of detection (ie, >5.4-log₁₀ in four trials and >3.7 log₁₀ in one trial). Complete viral inactivation was noted within 15 seconds.

Three trials of a phenolic revealed that a 1:10 dilution was incapable of inactivating more than approximately 2.0-log₁₀ of poliovirus, even with 10 minutes of contact time (Table 1). In two of the trials, essentially no inactivation was observed.

In four trials, a 1:10 dilution of a quaternary ammonium compound was able to achieve viral inactivation beyond the detection limit, with the exception of a single trial in which a 0.18-log₁₀ reduction was observed at 15 seconds.

Efficacy of Disinfectants Against Poliovirus in the Presence of Blood

In the presence of 80% blood, poliovirus inactivation by chlorine was: 5,000 ppm, 1- to 2-log₁₀; 500 ppm chlorine, 0.5-log₁₀; and 50 ppm, less than 0.2-log₁₀. Extending the contact time from 15 seconds to 10 minutes did not appreciably

increase the amount of viral inactivation. In the presence of 20% blood and 5,000 ppm chlorine, viral inactivation was beyond the limit of detection (>4.5-logs).

In the presence of 80% blood, a 1:10 dilution of a phenolic achieved essentially no poliovirus inactivation. In the presence of 20% blood, less than 1.1-log₁₀ of poliovirus were inactivated.

A quaternary ammonium compound inactivated less than 0.60-log₁₀ of poliovirus in the presence of 80% blood and less than 0.9 log₁₀ in the presence of 20% blood.

Because poor inactivation of poliovirus was observed for both the phenolic and the quaternary ammonium compound in the presence of blood using a 1:10 dilution, further experimental trials at the use dilutions of each product, 1:128, were not conducted.

Efficacy of Second Challenge of Hypochlorite Solutions Against Poliovirus

Three trials were conducted in which a second application of 5,000 ppm chlorine was added to the virus-blood mixture at 45 seconds (Table 2). In these trials, an increase of 1.1- to 4.3-log₁₀ inactivation was noted at the 1- or 2-minute sample compared to the 30-second sample. The initial inactivation of only 0.4 log₁₀ at 0.5 minute was increased to 1.4 and 2.7 log₁₀ after 0.25 and 1.25 additional minutes, respectively.

Efficacy of Disinfectants Against HSV in the Absence of Blood

In the absence of blood, complete inactivation of HSV-1 was achieved by all three disinfectants tested; hypochlorite at 5,000 ppm and 500 ppm chlorine, a phenolic at 1:10 and 1:128 dilutions, and a quaternary ammonium compound at 1:10 and 1:128 dilutions (Table 3).

Efficacy of Disinfectants Against HSV in the Presence of Blood

In the presence of 80% blood, 5,000 ppm chlorine was able to inactivate greater than 4-log₁₀ of HSV-1 by 15 seconds (Table 3). However, 500 ppm chlorine was able to inactivate less than 1-log₁₀ of HSV-1 within 10 minutes.

Both the phenolic and the quaternary ammonium compound at a 1:10 dilution were able to inactivate HSV-1 beyond the limits of detection within 30 seconds (Table 3). The quaternary ammonium compound at its use dilution, 1:128, was effective in inactivating greater than 3-log₁₀ of HSV-1 by 30 seconds. However, the phenolic at its use dilution of 1:128 was essentially ineffective in inactivating HSV-1.

DISCUSSION

Surface disinfection is practiced in the hospital to eliminate potential pathogens from environmental surfaces in order to decrease risks to healthcare workers (eg, removal of microorganisms from work surfaces in laboratories) and to eliminate the source for contamination of healthcare workers' hands (eg, terminal cleaning of rooms occupied by persons with vancomycin-resistant *Enterococcus* or methicillin-resistant *Staphylococcus*

TABLE 1
EFFICACY OF DISINFECTANTS AGAINST POLIOVIRUS TYPE I IN THE ABSENCE AND PRESENCE OF BLOOD

Disinfectant	Dilution	Exposure Time (min)	Mean Log ₁₀ Viral Reduction (Range)			
			80% Blood*†	20% Blood*†	Saline*†	
Hypochlorite	1:10 (5,000 ppm)	0.25	1.7 ₃ (1.2-2.1)	>4.5 ₁	>3.7 ₄ ‡	
		0.50	1.7 (1.2-1.9)	>4.5	>3.7	
		1.0	1.7 (1.2-1.9)	>4.5	>3.7	
		2.0	1.6 (1.2-1.9)	>4.5	>3.7	
		5.0	1.8 (1.3-2.1)	>4.5	>3.7	
		10.0	1.8 (1.4-2.0)	>4.5	>3.7	
	1:100 (500 ppm)	0.25	0.75 ₁	Not tested	>5.4 ₁	
		0.50	0.49	Not tested	>5.4	
		1.0	0.60	Not tested	>5.4	
		2.0	0.64	Not tested	>5.4	
		5.0	0.58	Not tested	>5.4	
		10.0	0.66	Not tested	>5.4	
	1:1,000 (50 ppm)	0.25	0.16 ₁	Not tested	Not tested	
		0.50	0.14	Not tested	Not tested	
		1.0	0.16	Not tested	Not tested	
		2.0	0.11	Not tested	Not tested	
		5.0	0.12	Not tested	Not tested	
		10.0	0.04	Not tested	Not tested	
	Phenolic	1:10	0.25	NC ₂ (0.0)§	0.78 ₁	0.68 ₃ (2.0)§
			0.50	NC (0.0)§	0.93	0.40 (1.9)§
1.0			NC (0.03)§	0.76	0.37 (2.0)§	
2.0			0.21 (0.81)§	0.75	0.41 (0.75)§	
5.0			NC§	0.77	0.40 (0.77)§	
10.0			NC§	1.07	0.42 (1.07)§	
Quaternary ammonium compound	1:10	0.25	0.56 ₂ (0.51-0.60)	0.81 ₁	0.18 ₁ ¶	
		0.50	0.14 (0.07-0.20)	0.78	>2.4¶	
		1.0	0.22 (0.13-0.30)	0.78	>2.4¶	
		2.0	0.09 (0.07-0.11)	0.71	>2.4¶	
		5.0	0.09 (0.04-0.14)	0.77	>2.4¶	
		10.0	0.10 (0.04-0.15)	0.83	>2.4¶	

Abbreviations: NC, no change.

* Subscripts refer to number of experimental trials conducted (applies to all exposure times for that blood mixture and disinfectant dilution).

† Values represent means of experimental runs; when viral reduction exceeded detection limits a ">" sign is shown and the most conservative (ie, lowest) viral reductions among the trials is reported.

‡ Detection limits of the four experimental runs were >3.7, >5.4, >5.4, and >5.6.

§ An increase in virus (maximum 0.31-log₁₀) was detected; if the upper range of viral concentration detected indicated an increase in virus, only the lower range was listed.

¶ All other runs were above the detection limits (ie, >4.6, >4.6, >5.4).

¶¶ Detection limits of the four experimental runs were >2.4, >4.6, >4.6, >5.4.

aureus). Desirable characteristics of disinfectants used on environmental surfaces include broad antimicrobial spectrum, rapid action, lack of toxicity, material comparability, user safety, and minimal interference by protein or blood. Some disinfectants have limited use, because they do not meet some of these criteria, including flammability (ie, alcohols) and irritating vapors (ie, glutaraldehydes). Commonly used surface disinfectants include hypochlorite (100 ppm chlorine), phenolics (use dilution), quaternary ammonium compounds (use dilution), ethyl or isopropyl alcohol (70%-90%), and iodophor germicidal detergent solution (use dilution).¹¹

Factors affecting the efficacy of individual disinfectants include concentration, contact time, presence of salt

or protein, pH, temperature, humidity, surface composition, and type of pathogen. Environmental surfaces are considered noncritical items in the Spaulding classification, and hence low-level disinfectants are recommended (ie, hospital disinfectant without label claim for tuberculocidal activity).¹¹ OSHA requires that "contaminated work surfaces shall be decontaminated with an appropriate disinfectant after completion of procedures . . . or after any spill of blood or other potentially infectious material."⁹ The CDC recommends that 5.25% sodium hypochlorite (household bleach) at an initial concentration of 500 ppm (1:100 dilution) be used for the decontamination of a blood spill.¹⁰

When chlorine reacts with proteinaceous material, such as blood, some of the chlorine combines with proteins

TABLE 2
EFFICACY OF TWO DOSES OF HYPOCHLORITE AGAINST POLIOVIRUS TYPE I IN THE ABSENCE AND PRESENCE OF BLOOD

Disinfectant	Dose	Total Exposure Time (min)	Exposure Time, Dose 2 (min)	Mean Log ₁₀ Viral Reduction* (Range)	
				80% Blood*†	Saline*†
Hypochlorite (1:10 dilution)	1	0.25	—	0.35 (0.02-0.51)	>5.3
	1	0.50	—	0.38 (0.19-0.66)	>5.3
	2	1.0	0.25	1.4 (0.64-2.4)	>5.3
	2	2.0	1.25	2.7 (1.1-4.7)	>5.3
	2	5.0	4.25	2.9 (1.5-4.9)	>5.3
	2	10.0	9.25	2.9 (1.5-4.9)	>5.3

* Mean of three experimental trials.

† Values represent means of experimental runs; when viral reduction exceeded detection limits, a ">" sign is shown and the most conservative (ie, lowest) viral reductions among the trials is reported.

TABLE 3
EFFICACY OF DISINFECTANTS AGAINST HERPES SIMPLEX VIRUS TYPE I IN THE ABSENCE AND PRESENCE OF BLOOD

Disinfectant	Dilution	Exposure Time (min)	Mean Log ₁₀ Viral Reduction (Range)	
			80% Blood*†	Saline*†
Hypochlorite	1:10 (5,000 ppm)	0.25	>4.4 ₂	>4.6 ₂
		0.50	>4.4	>4.6
		1.0	>4.4	>4.6
		2.0	>4.4	>4.6
		5.0	>4.4	>4.6
		10.0	>4.4	>4.6
Hypochlorite	1:100 (500 ppm)	0.25	0.27 ₂ (0.06-0.47)	>4.6 ₂
		0.50	0.29 (0.05-0.52)	>4.6
		1.0	0.29 (0.04-0.53)	>4.6
		2.0	0.32 (0.17-0.47)	>4.6
		5.0	0.35 (0.12-0.58)	>4.6
		10.0	0.37 (0.19-0.54)	>4.6
Phenolic	1:10	0.50	>2.5 ₂	>2.6 ₂
		1.0	>2.5	>2.6
		2.0	>2.5	>2.6
		5.0	>2.5	>2.6
		10.0	>2.5	>2.6
Phenolic	1:128	0.50	0.18 ₂ (0.14-0.22)	>3.8 ₂
		1.0	0.20 (0.09-0.30)	>3.8
		2.0	0.00 (+0.06-0.06)	>3.8
		5.0	0.12 (0.04-0.20)	>3.8
		10.0	0.56 (0.012-1.0)	>3.8
Quaternary ammonium compound	1:10	0.50	>2.5 ₂	>2.5 ₂
		1.0	>2.5	>2.5
		2.0	>2.5	>2.5
		5.0	>2.5	>2.5
		10.0	>2.5	>2.5
Quaternary ammonium compound	1:128	0.50	>3.4 ₂	>3.4 ₂
		1.0	>3.4	>3.4
		2.0	>3.4	>3.4
		5.0	>3.4	>3.4
		10.0	>3.4	>3.4

* Subscripts refer to number of experimental trials conducted (applies to all exposure times for that blood mixture and disinfectant dilution).

† Values represent means of experimental runs; when viral reduction exceeded detection limits, a ">" sign is shown and the most conservative (ie, lowest) detection limit among the runs is reported.

and forms N-chloro compounds. It is for this reason that a high concentration of available chlorine is required to inactivate virus in the presence of undiluted blood. Interestingly, the contact time did not appreciably alter the effectiveness of the disinfectants against poliovirus for any product or at any concentration of chlorine. Fifteen seconds of contact produced a similar degree of inactivation as 10 minutes. In the presence of 80% blood, the addition of a second chlorine challenge at 45 seconds resulted in an increased elimination of poliovirus by 1- to 4-log₁₀.

Several studies have reported the efficacy of disinfectants against HIV¹⁴⁻¹⁹ and HBV.^{20,21} In the absence of blood, the following disinfectants inactivated greater than 3-log₁₀ of HIV by 1 minute: 70% ethanol,^{17,19} hypochlorite at concentrations from 50 to 5,000 ppm,^{15,16,18,19} a phenolic,¹⁹ and a quaternary ammonium compound.¹⁹ Some studies have reported cell-associated virus to be more resistant to disinfectants,^{18,19} whereas others have demonstrated that cell-free virus is more resistant.¹⁷ When dried onto glass slides, the efficacy of 70% ethanol against HIV has been reported to be reduced by some,^{17,22} but not all, investigators.¹⁹ HIV suspended in blood has been reported to be less susceptible to disinfectants. Although hypochlorite 5,000 ppm has been reported to inactivate HIV within 2 minutes in the presence of blood,¹⁶ other investigators have reported that 5,000 ppm¹⁸ and 2,500 ppm were ineffective.¹⁹ Other disinfectants reported to be unable to inactivate HIV within 1 minute in the presence of blood included a quaternary ammonium compound and a phenolic.¹⁹ However, 70% ethanol has been reported to be effective even in the presence of blood.^{18,19} Phenolics and quaternary ammonium compounds were able to inactivate HBV within 10 minutes,²¹ and 3,180-ppm chlorine was found to be effective within 2 minutes.²⁰ We are unaware of any published reports of studies that evaluated the effect of blood on the virucidal activity of disinfectants against HBV. There is only one published report that evaluates the efficacy of disinfectants against HCV. It shows that phenolic disinfectants inhibit HCV binding and replication, but chlorine was less effective (probably as a result of its low concentration in the presence of protein substances).²³

HIV is capable of retaining infectivity in dried 2% fetal bovine serum for 5 days²⁴ and in dried blood for more than 3 days.²⁵ HBV is capable of retaining its infectivity in dried blood for at least 1 week.²⁶ Treatment with the following disinfectants has been shown to inactivate HBV in dried blood: hypochlorite (500 mg free chlorine/mL) with 10-minute contact time, 70% isopropyl alcohol, and an iodophor disinfectant (80 mg available iodine/L).²⁷

Since poliovirus is more resistant to disinfectants than HIV and HBV, our data suggest that, in the absence of blood, the following disinfectants would be capable of inactivating HIV and HBV: hypochlorite at a final concentration of 5,000 ppm (1:10 dilution) and 500 ppm (1:100 dilution), a phenolic at its use dilution, and a quaternary ammonium compound at its use dilution. In the presence of 80% blood, hypochlorite at a final concentration of 500 ppm (1:100 dilution) and a phenolic at its use dilution were unable to inac-

tivate HSV-1 completely. Because HSV-1 has similar susceptibility to disinfectants as HIV, phenolics at their use dilution and 1:100 diluted hypochlorite are unlikely to inactivate HIV or HBV reliably. Chlorine at 5,000 ppm (1:10 dilution) inactivated HSV-1, and therefore our data suggest that HIV and HBV are also likely to be inactivated, provided that the final concentration of chlorine is at least 5,000 ppm. Our data demonstrated that a quaternary ammonium compound was effective in inactivating HSV and hence likely to be effective in inactivating HIV in the presence of blood. However, we tested the quaternary ammonium compound only at its use dilution, and, when mixed with blood, an additional dilution effect would occur. Since we did not test less diluted preparations, we are unable to predict the efficacy of these compounds for inactivating HIV when suspended in liquids. Furthermore, we tested only a single phenolic and quaternary ammonium compound, and our results should not be generalized to all products, as efficacy may vary among different types or brands of phenolics and quaternary ammonium compounds.²⁸

Our data suggest that prolonging the contact time from 15 to 30 seconds to 10 minutes did not appreciably improve virucidal activity. Although adding a second dose of hypochlorite at 45 seconds improved the virucidal efficacy of hypochlorite in the presence of 80% blood, complete inactivation of poliovirus was not achieved, and hence this procedure cannot be recommended to guarantee complete inactivation of HIV or HBV at this time.

In conclusion, our data suggest that environmental surfaces without visible blood may be disinfected with a diluted hypochlorite solution, a phenolic, or a quaternary ammonium compound. Both diluted hypochlorite (1:10, 1:100) and the quaternary ammonium compound had superior antiviral activity when compared to the phenolic, but our data suggest that phenolics likely are effective against HIV and HBV in the absence of blood.

Based on these experiments and previously reported studies, we believe that healthcare workers should assume that blood spills treated with diluted hypochlorite solutions potentially contain infectious viruses. Such spills should be cleaned using care to avoid contaminated percutaneous injuries from glass or sharps and mucous membrane exposure to blood. As recommended by the CDC, workers should wear gloves while cleaning blood spills.¹⁰ If splashing is anticipated, protective eyewear should be worn, along with an impervious gown or apron that provides an effective barrier to splashes. Sharps or broken glassware should not be picked up directly with the hands; they should be cleaned up using mechanical means (eg, brush and dustpan, tongs, or forceps). Large blood spills should be treated initially with hypochlorite so as to achieve a final concentration of 5,000-ppm chlorine (ie, 1:10 dilution). For large spills, this will entail using undiluted bleach, because the very act of adding sodium hypochlorite to a contaminated fluid will result in dilution proportional to the size of the spill and the amount of hypochlorite added. Even if treatment with hypochlorite does not completely inactivate all potential viral pathogens, substantial reduction of infec-

tious viruses will occur, thereby reducing the risk of disease acquisition in the event of healthcare worker injury. Following cleanup of large spills, the environmental surface should again be disinfected. CDC guidelines¹⁰ that recommend using an initial hypochlorite concentration of 500 ppm (1:100 dilution) chlorine may not provide adequate virucidal activity and should be modified to recommend a higher concentration of 5,000 ppm (1:10 dilution). For surfaces contaminated with small amounts of blood (ie, a few drops), we believe that the area may be cleaned and disinfected in a single-step procedure using an absorbent cloth or mop soaked with an appropriate disinfectant.

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