ARTICLES

Thirty-nine products representing six categories of disinfectants (alcohols, chlorines, dilute glutaraldehydes, iodophors, phenolics, and quaternary ammonium compounds) were first tested in the absence of bioburden, using four test methods with five test organisms. Products that performed best were retested with the same methods and organisms in the presence of both serum and whole blood, using 3- and 10-minute contact times. Only products containing high ethyl alcohol had consistently high antimicrobial activity regardless of the test method, test organism, or contact time used both in the absence and presence of bioburden. Although these specific formulations demonstrated ability to penetrate and inactivate high concentrations of microorganisms within heavy bioburden, optimum disinfection of environmental surfaces is highly formulation dependent. Other products tested showed deficiencies that contraindicate their use as environmental surface disinfectants in clinical dental settings.

Antimicrobial activity of environmental surface disinfectants in the absence and presence of bioburden

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urrent methods of dental treatment cause widespread contamination of the operating area. 1-8 Droplet splatter, flying debris, contaminated hands and instruments, and operation of automated instruments such as handpieces, ultrasonic scalers, and air-slurry polishers contribute to the problem. 1-3,9-20

The potential of contaminated environmental surfaces to transmit infection continues to be questioned by some investigators. However, confirmation of long-term survival of large numbers of pathogenic organisms on a variety of surfaces²¹⁻³⁵ has made infection via

contaminated surfaces credible, and experimental data corroborate the possibility. For example, both rotavirus and rhinovirus have been transferred from contaminated hands, to objects, and to clean hands in sufficient numbers to elicit infection in susceptible humans.36-37 Furthermore, colds caused by rhinovirus have been transmitted to human volunteers experimentally via contaminated ceramic cup handles and plastic tiles.38 Comparable correlations at the clinical level have been difficult because so much time elapses between infection and overt symptoms that subjects often cannot recall objects contacted. Even when recall is possible, the incubation time lapse can exceed organism survival time on inanimate surfaces, and culturing becomes impossible. Therefore, clinical reports linking infection with objects have been based on circumstantial evidence. Examples include epidemiologically linked diseases,^{21,39-44} spread of infection in facilities in which direct contact of infected subjects was impossible,⁴⁵⁻⁴⁶ clinical experiments,⁴⁷ and documentaries.⁴⁸ Reports such as these make it impossible to rule out environmental surfaces as fomites.

Until data become available to demonstrate conclusively that contaminated

 Table 1 ■ Alphabetical listing by brand name of all disinfectants tested. The far right column indicates the concentration of active ingredients at the recommended use-dilution listed on the label.

BRAND NAME		COMPANY	BATCH NUMBER	RECOMMENDED DILUTION	USE CONCENTRATION OF ACTIVE INGREDIENT(S)	
1. BASIC-G		Shaklee	W6F1	1:256	0.05% quaternary ammonium compounds 0.01% tetrasodium EDTA 0.001% essential oils	
2. BIOCIDE		Biotrol Inc	Not available	1:213	82 ppm available iodine	
3. BIOCIDE		Biotrol Inc	Not available	1:106 *	164 ppm available iodine	
4. BORAXO		U.S. Borax	5099E06	Use as packaged	9.13% tetrasodium EDTA 5.0% isopropyl alcohol 0.07% 5-chloro-2-(2,4-dichlorophenoxy) phenol	
5. CITRACE		Caltech Industries, Inc	FJ86 1007	Use as packaged	66.6% ethyl alcohol 0.12% o-phenylphenol	
6. CLOROX		The Clorox Co	U6094R	1:5	1.05% sodium hypochlorite	
7. CLOROX		The Clorox Co	U6094R	1:10	0.52% sodium hypochlorite	
8. CLOROX		The Clorox Co	U6094R	1:20	0.26% sodium hypochlorite	
9. CLOROX FRESH S	CENT	The Clorox Co	Not available	1:5	1.05% sodium hypochlorite	
10. CLOROX FRESH S	CENT	The Clorox Co	Not available	1:10	0.52% sodium hypochlorite	
11. CLOROX FRESH SO	CENT	The Clorox Co	Not available	1:20	0.26% sodium hypochlorite	
12. COESPRAY	••••••	Coe Laboratories, Inc	1366 L21T0656	Use as packaged	53.46% ethyl alcohol 0.9% essential oils 0.176% o-phenylphenol 0.044% p-tert-amylphenol	
13. DENTASEPTIC		Heraeus Dental Gold	20J5M54	1:32	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol	
14. DISPATCH		Caltech Industries, Inc	FJ870121	Use as packaged	0.55% sodium hypochlorite	
15. ETHYL ALCOHOL	(non-denatured)	Midwest Grain Products	Not available	Use as packaged	70% v/v non-denatured ethyl alcohol	
16. ETHYL ALCOHOL	(denatured)	Whiteworth, Inc	1711606	Use as packaged	70% v/v denatured ethyl alcohol	
17. ETHYL:ISOPROPYL	ALCOHOL MIX.	Midwest Grain Products &	Not available	Experimental	40% ethyl alcohol 40% isopropyl alcohol	
18. ETHYL:ISOPROPYL	ALCOHOL MIX.	Midwest Grain Products &	Not available	Experimental	45% ethyl alcohol 45% isopropyl alcohol	
19. EXSPOR		Fisher Scientific	BX023	1:1:4	1.43% organic acid 0.23% sodium chloride	
20. HIBISTAT TOWELE	TTE	Stuart Pharmaceuticals	9034B	Use as packaged	70% isopropyl alcohol 0.5% chlorhexidine gluconate	
21. HI-TOR		Huntington Laboratories, Inc	01-03	1:256	0.06% quaternary ammonuim compounds	
22. ISOPROPYL ALCOI		Fisher Scientific	865740	Use as packaged	70% v/v isopropyl alcohol	
23. LYSOL LIQUID (Pine		Lehn & Fink Products	IL 14115	1:103	0.16% soap	
ES. ETGOL EIGGID (FIII)	o ocenity				0.05% pine oil 0.04% o-benzyl-p-chlorophenol 0.015% isopropyl alcohol 0.007% tetrasodium EDTA	
24. LYSOL SPRAYS (Regular Scent, Fre Light Scent, Profes	sh Scent,	Lehn & Fink Products	IL 16116	Use as packaged	79% ethyl alcohol 0.1% o-phenylphenol	
25. MATAR		Huntington Laboratories, Inc	E5-12	1:256	0.04% o-benzyl-p-chlorophenol 0.02% o-phenylphenol 0.01% isopropyl alcohol 0.01% p-tert-amylphenol 0.007% tetrasodium EDTA	
26. MULTICIDE		Biotrol, Inc.	Not available	1:32	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol	
27. OMNI II		ADM Medical Division	11C3M6	1:32	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol	
28. PERMACIDE 18		Sporicidin International	Not available	Use as packaged	18% ethyl alcohol 18% isopropyl alcohol	
29. PERMACIDE 30		Sporicidin International	Not available	Use as packaged	30% ethyl alcohol 30% isopropyl alcohol 0.37% o-phenylphenol	
30. PRECISE 31. PRESEPT LIQUID.		Surgikos, Scotland	VC003	Use as packaged	70% v/v ethyl alcohol B 1.25% w/v chlorhexidine gluconate 0.1% w/v quaternary ammonium compound	
32. PRESEPT TABLET 33. PRESEPT WIPE		Surgikos, U.S.A	1089	1:250 Use as packaged	1000 ppm available chlorine 70% denatured ethyl alcohol 1.25% chlorhexidine gluconate	
34. PROCIDE ES		Cottrell Ltd	2036 1M31 T 1309	Use as packaged	0.1% quaternary ammonium compounds 52.79% ethyl alcohol 0.176% o-phenylphenol 0.044% p-tert-amylphenol	
35. SPORICIDIN SPRA	ν	Sportcidin International	211M 1	Use as packaged	18.0% ethyl alcohol 1.41% phenol 0.25% essential oils 0.24% sodium phenate	
36. STERALL SPRAY.		Colgate-Hoyt	6C52	Use as packaged	1.50% triethylene-glycol 0.25% glutaraldehyde	
37. VITAWIPES		Block Professional Dental Products Co	11719	Unknown	10.28% quaternary ammonium compounds	
38. WESCODYNE		AMSCO Medical Products	Not available	1:213	75 ppm available iodine	
9. WESCODYNE		AMSCO Medical Products	Not available	1:106*	150 ppm available lodine	

^{*} Tested at twice manufacturer's suggested strength.

environmental surfaces cannot transmit infections, clinicians cannot ignore or treat them lightly. As dental operatories have innumerable environmental surfaces that are contaminated during routine patient treatment, the effectiveness of products used to disinfect these surfaces must be examined. This investigation was conducted to test a number of commercial products to identify those with broad-spectrum, rapid antimicrobial activity both in the absence and presence of bioburden.

Methods and materials

The general protocol specified use of four test methods and five test organisms with 39 disinfectants in the absence of bioburden. Disinfectants demonstrating best antimicrobial activity under these conditions were then tested with the same methods and organisms in the presence of bioburden.

Disinfectant selection and preparation

Table 1 lists the disinfectants selected for this study based on a product-use survey, 49 manufacturers' communications, and literature review. All products were prepared according to manufacturers' directions immediately before testing. When dilution was specified, sterile deionized water was used.

Test organism selection and preparation

Test organisms were: Pseudomonas aeruginosa ATCC 15442, Salmonella choleraesuis ATCC 10708, Staphylococcus aureus ATCC 6538, Mycobacterium bovis (BCG) ATCC 35743, and poliovirus type I (Mahoney strain). The four bacteria were selected because they are specified as test organisms by the Environmental Protection Agency (EPA) to register hospital disinfectants⁵⁰ and to establish tuberculocidal claims.⁵¹ Poliovirus I was selected because it is resistant to inactivation by many disinfectants.⁵²⁻⁵⁴

Bacteria were prepared according to EPA specifications, except stock cultures were stored in liquid nitrogen. Poliovirus was grown in HeLa cells, harvested by multiple freeze/thaw cycles and cesium chloride (CsCl) banding, and stored at 4 C in CsCl.

Test methods

Association of Official Analytical Chemists Use Dilution Method (AOAC *UDM*). The standard AOAC UDM, using 60 stainless steel penicylinders per replicate, was performed precisely as described in AOAC literature.⁵⁰ Secondary subculture was performed on each carrier and both subcultures were incubated at 37 C for 48 hours. For bioburden testing, the same method was used except penicylinders were coated with a 50:50 vol/vol mixture of human whole blood and bacterial culture.

Environmental Protection Agency Tuberculocidal Activity Test Method (EPA TB ATM). The standard quantitative EPA TB ATM was performed precisely as specified in EPA literature.⁵¹ For bioburden testing, the same method was used except test suspensions contained 50% horse serum which delivered a 5% concentration of horse serum to the disinfectant.

Virucidal Suspension Test. A suspension test, rather than a carrier method, was used for all virucidal testing because all attempted procedures for drying virus onto carrier surfaces, and subsequent virus recovery, caused loss of viral titer that was unpredictable and unacceptably high (> 2 log₁₀ units). Tests performed without blood used 100 μ L of virus, $\geq 10^6$ plaque-forming units (PFU), added to 900 µL of disinfectant. After prescribed contact times, phosphate-buffered saline solution was used for serial 1:10 dilutions. and four consecutive dilutions of virus were assayed in duplicate on monolayers of HeLa cells for infectious poliovirus. Twelve-well plates (12-Well Tissue Culture Cluster 3512, Costar) were incubated at 37 C for 48 hours and stained. Log₁₀ reductions were calculated from plaque counts. Disinfectants yielding three log₁₀ reductions in virus titer were evaluated for interference with virus attachment to HeLa cells. Each 12-well plate included controls to assay for titer of viral challenge and test for system contaminants. Also, disinfectant cytotoxicity to HeLa cells was determined. In cases in which cytotoxicity was detected, dilution was used to eliminate this effect. Dilution was also used to eliminate the effect of residual disinfectant on the virus in the assay system.

Testing with human whole blood used the same procedures described except $10 \,\mu\text{L}$ of poliovirus ($\geq 10^6 \,\text{PFU}$) was added to $100 \,\mu\text{L}$ of blood and allowed to stand for 1 minute before $900 \,\mu\text{L}$ of disinfectant was added. Controls were included to determine amount of virus inactivated by blood. For tests in which urea was included to disrupt ethyl alcohol-induced



Fig 1

Appearance of the test tray used in the CRA EWM. This test was designed to mimic dental clinical procedures used to disinfect contaminated operating sites.

blood aggregates, the same procedures were employed except that siliconized tubes (Sigmacote, Sigma Chemical) were used and 9.0 mL of 7.0 mol/L urea was added to the blood-virus-disinfectant mixture after prescribed contact times. Log₁₀ reductions were calculated for all work involving poliovirus (both with and without bioburden) using the formula: log₁₀ reduction = log₁₀ (titer of viral challenge per mL) - log₁₀ (titer infectious virus per mL after exposure to disinfectant).

Clinical Research Associates Environmental Wipe Method (CRA EWM). A test was devised to mimic dental clinical procedures used for disinfection of environmental surfaces to determine if wiping with disinfectant-soaked gauze sponges inactivated organisms dried onto surfaces, both in the absence and presence of bioburden. Silicone adhesive (Mirror 3 Tray Adhesive, Kerr/Sybron) and caulking (Silicone II GE5070, General Electric Co) were used to attach an 81/16-× 11⁷/₈-in piece of laminated plastic counter covering (1595-6 Black Wilsonart, Ralph Wilson Plastic Co) to 84- × 12in polypropylene trays (Size B Trays 20Z401, Zirc Dental), which were trimmed to fit snugly under their polyethylene lids (Tray Cover 20Z441, Zirc Dental). Figure 1 shows the test tray after construction and before inoculation with test organisms. Lids and trays were sterilized with ethylene oxide (ETO) for 2 hours at 135 F and aerated for 8 hours before 2 mL of bacterial suspension was applied with a sterile 2- × 2-in cottonfilled gauze sponge (Cotton Filled Sponges 6000207, Healthco). After the organisms applied to the trays were completely dried (20-30 minutes) in a laminar flow hood (NU-408FM-600, Nuaire), 3.5 mL of disinfectant was pipetted onto a sterile gauze sponge which was then used to wipe the test surface for 10 seconds using about 150-g pressure with overlapping strokes (20 left to right, followed by 20 top to bottom). Disinfectants sold in aerosol spray cans were treated in the same manner to standardize the amount of disinfectant delivered to the contaminated surface by spraying the disinfectant into a sterile test tube before pipetting. After wiping, the disinfectants were left on the trays for 3 minutes before one of two methods was used to determine the number of viable organisms remaining on the tray. The method depended on the test organism.

M bovis coated travs were flooded with 50 mL of tryptic soy broth (TSB) with neutralizers (ingredients are enumerated in section on media and neutralizers), and scrubbed for 1 minute with a sterile polypropylene brush (Nail Brush 501, Kellogg Brush Co) to remove and suspend viable organisms. The fluid was collected and diluted, and duplicate 1-mL samples of each dilution, plus the undiluted fluid (approximately 40 mL), were passed through 0.45-µm filters. Each filter was washed twice with 100 mL of sterile 0.1% peptone water, placed on Mycobacterium 7H11 agar, and incubated at 37 C for 21 days. To allow calculation of log10 reductions, trays wiped with water were included in all tests. Log₁₀ reductions were calculated using the formula: log₁₀ reduction = log_{10} (number of viable organisms from water control tray) log₁₀ (number of viable organisms from test tray).

P aeruginosa, S choleraesuis, and S aureus remaining viable organisms were assayed directly on the trays by adding 300 mL of tryptic soy agar (TSA) with neutralizers at 45 C. Trays were covered with their lids and incubated at 37 C for 48 hours. Colonies were counted, with > 500 designated as "too numerous to count" (TNC). Trays wiped with water were included as organism viability controls. Results of this testing were reported two ways: by the number of colony forming units (CFU) for each of the three test bacteria that survived disinfectant treatment, and as percent of tests less than TNC. To determine the percent of tests less than TNC for a particular disinfectant, the following formula was used: percent of tests less than TNC = number of tests less than TNC for the three organisms ÷ total number of tests × 100. To determine the mean percent of tests less than TNC for a category of disinfectants with the same active ingredient, the mean of all disinfectants within the category was calculated.

The CRA EWM bioburden testing with all four bacteria used the same methods, except cultures were mixed 50:50 vol/vol with human whole blood; 1 mL of this mixture was spread on trays with a sterile glass rod. For tests with S aureus and M bovis in which urea was included to disrupt blood aggregates, the procedures described previously for M bovis were used except that 50 mL of 7.0 mol/L urea was substituted for TSB used to suspend organisms from the surface.

Media and neutralizers

For M bovis, TSB (Difco) containing 1% Tween 80 (Fisher Scientific), 1% lecithin (Sigma), and 0.4% sodium thiosulfate (Sigma) were used in CRA EWM tests, and Mycobacterium 7H11 agar (Difco) was used for all subcultures. For P aeruginosa, S choleraesuis, and S aureus, TSA (TSB + 1.5% Bacto-agar, Difco) containing 0.5% Tween 80, 0.1% lecithin, and 0.1% sodium thiosulfate was used as the subculture medium. Minimum Essential Medium (Irvine Scientific) supplemented with 10% calf bovine serum enriched with iron (HyClone) was used for HeLa cell cultures.

Selection of bioburden and procedures used for testing with bioburden

Because of its clinical relevance, human whole blood was used as the bioburden challenge whenever possible. A concentration of 50% human whole blood in the blood-bacterial suspension mixture was used with the AOAC UDM and CRA EWM tests performed with P aeruginosa, S choleraesuis, and S aureus. A 10% concentration of human whole blood was used with virus suspension tests because higher concentrations precluded proper mixing and pipetting with some disinfectants. Two different types of bioburden were used with M bovis to accommodate test method differences. A 50% concentration of human whole blood in the bacterial suspension was used in the CRA EWM tests with this organism, and 5% horse serum in the bacterial suspension-disinfectant mixture was used in the EPA TB ATM tests. Five percent horse serum was used because it has been specified as the bioburden

for other standard EPA tests.55

Use of human whole blood as the bioburden required special procedures when ethyl alcohol-containing disinfectants were used because they caused the organism-media-whole blood mixture to form aggregates. Different chemicals were sought to disperse the aggregates without affecting viable organisms, and 7.0 mol/L urea met the criteria best.56 Although 7.0 mol/L urea destroyed gramnegative bacteria, it allowed almost complete recovery of M bovis, S aureus, and poliovirus. Therefore, when ethyl alcohol disinfectants were tested with whole blood bioburden, the CRA EWM tests using S aureus and M bovis and suspension tests using poliovirus included the addition of 50 mL and 9 mL of 7.0 mol/L urea, respectively, after disinfectant treatment. The same tests were performed without urea so results could be compared.

Selection of wipe material

As it has been reported that cotton may interfere with the antimicrobial activity of iodophors,8 this was also evaluated. For this test, sterile GSA centrifuge bottles containing 64 mL each of Biocide and Wescodyne iodophors diluted 1:213 received 8 g of three different wipe materials (Cotton-Filled Gauze Sponges by Healthco, Nu-Gauze rayon/polyester sponges by Johnson and Johnson, and Viva Paper Towels by Scott Paper Co). After 10 minutes, samples were centrifuged for 20 minutes at 6,000 × g. An aliquot of 9.9 mL was removed from each GSA bottle and 0.1 mL of S aureus culture was added. Assays for viable organisms were performed at 3 minutes.

Tests for chemical interference and neutralizer efficacy

To assay for possible toxic effects of ETO residuals or materials, or both, used in the CRA EWM, 300 mL of TSA with neutralizers containing about 100 CFU was poured into an ETO sterilized CRA EWM tray, a CRA EWM tray disinfected with 70% vol/vol denatured ethyl alcohol and not ETO sterilized previously, and several large sterile petri plates (150×15 mm). Tests used three replications each of *P aeruginosa*, *S choleraesuis*, and *S aureus*.

To test efficacy of the neutralizers in TSB, 1 mL of each disinfectant was added to 9 mL of TSB with neutralizers. After 1 minute, about 100 CFU of *M bovis*

Table 2 ■ Colony and plaque counts from tests of 39 disinfectants using four test methods with five test organisms in the absence of bioburden. Tinted columns show results and white columns list the number of test replications. Low numbers in the tinted columns indicate good antimicrobial activity.

DISINFECTANTS	Мус		rium I	bovis (1 743)	BCG)	(M		virus I ey strai	in)			15442		PA		cholera 10708)				ccus au 6538)	ireus
		EPA T	в атм	CRA	EWM	VIR	us su	SPENS	ION	AOAC	UDM	CRA	EWM	AOAC	UDM	CRA	EWM	AOAC	UDM	CRA	EWM
urst valen ett 5	TB claim	result	Reps (n)	3 min result Ctu §	Reps	3 min result Pfu §§	Reps (n)	10 min result Pfu §§	Reps	10 min result	Reps (n)	3 min result Ctu §		10 min result #	Reps (n)	3 min result Clu §		10 min result	Reps (n)	3 min result Cfu §	Reps (n)
I. ALCOHOLS																					
A. Ethyl Alcohol 1. Citrace	10 min	4P	(4)	1	(6)	0	(7)	٥	(4)	2P	(2)	O	(3)	2P	(2)	0	(3)	2P	(2)	0	(3)
2. CoeSpray † 3. Ethyl, 70% v/v	10 min none		(4)	53	(6) (4)	0	(6) (10)	0	(3)	2P 2P	(2)	0	(3)	2P 2P/1F	(2)	0	(3)	2P 2P	(2)	1	(3)
non-denatured														886				2P			
4. Ethyl, 70% v/v denatured	none		(4)	7	(6)	0	(10)	0	(6)	2P	(2)	0	(3)	2P	(2)	0	(3)		(2)	Đ	(3)
5. Lysol sprays (Fresh Scent, Regular Scent, Light Scent,	10 min	AP.	(4)	2	(7)	0	(6)	0	(3)	2P	(2)	0	(3)	2P	(2)	0	(3)	2P	(2)	1	(3)
Professional) 6. Presept Liquid 7. Presept Wipes 8. ProCide ES	none none 10 min	4P - 4P	(4) - (4)	Z TNC 6	(6) (5) (6)	0 0	(5) — (5)	0 -0	(3)	- - 2P	- (2)	TNC 0	(3) 3 (3)	 2P	_ (2)	6	(3) (3) (3)	- - 2P	_ (2)	0 0	(3) (3) (3)
B. Isopropyl Alcohol				2	(3)	TNC	(4)	TNC	(2)			0	(3)	_		0	(3)			1	(3)
9. Hibistat Towelette liquid 10. Isopropyl, 70% v/v	none	4P	(4)	3	(3)	TNC	(6)	TNC	(2)	2P	(2)	D	(3)	2P	(2)	a	(3)	2P	(2)	1	(4)
C. Isopropyl-Ethyl Alcohol Mixture																					
11. 40%:40% v/v 12. 45%:45% v/v	none	3P 3P	(3)	9	(3)	TNC	(4) (4)	TNC	(3)	2P 2P	(2) (2)	0	(3)	2P 3P	(2)	TNC	(3)	2P 2P	(2) (2)	0 23	(3)
13. Permacide 18	none	4P	(4)	58	(3)	TNC	(4)	TNC	(3)		-	0	(3)	=	-	0	(3)	=	-	0	(3)
14. Permacide 30	none	4P	(4)	6	(3)	FINE	(3)	TNC	(2)	-		U	(3)				(3)	1	_	0	(3)
. CHLORINES													(0)	a.b.	(0)		100	200	(0)		(0)
15. Clorox 1:5 16. Clorox 1:10	none	4P 3P	(4)	3 38	(9)	0	(7)	0	(4)	3P 3P	(3)	0 4	(3)	3P 3P	(3)	1 0	(3)	3P 3P	(3)	2	(3)
17. Clorox 1:20 18. Clorox Fresh	none	4P 4P	(4) (4)	184 35	(7)	0	(3)	0	(4)	2P 2P	(2)	0	(3)	2P 2P	(2)	0 2	(3)	2P	(2)	0	(3)
Scent 1:5		3P/1F	(4)	24	(3)	0	(3)	0	(2)	3P	(3)	0	(3)	3P	(3)	0	(3)	3P	(3)	19	(3)
19. Clorox Fresh Scent 1:10																				0	1000
20. Clorox Fresh Scent 1:20	none	1	(3)	45	(3)	0	(4)	0	(2)	1P/2F	(3)	1	(3)	2P	(2)	1	(3)	2P/1F	(3)		(3)
21. Dispatch 22. Exspor 1:1:4	10 min		(4)	302 TNC	(6) (6)	0	(8)	0	(3)	2P 2P	(2)	0	(3)	2P 2P	(2)	0	(3)	2P 2P	(2)	0	(3)
23. Presept Tablets 1:250	none	4P	(4)	TNC	(5)	0	(3)	6.	(3)	28	(2)	0	(3)	2P	(2)	-2	(3)	2P	(2)	236	(3)
II. GLUTARALDEHYDE 24. Sterall Spray †	30 min	4F	(4)	TNC	(3)	TNC	(3)	TNC	(3)	2PITF	(3)	8	(3)	2P	(2)	0	(3)	2P	(2)	1	(3)
					- 11																
V. IODOPHORS 25. Biocide 1:213	10 min	5P	(5)	TNC	(6)	O	(8)	0	(3)	1P/2F	(3)	0	(3)	2P/1F	(3)	1	(3)	3P/1F	(4)	THE	(4)
26. Biocide 1:106 27. Wescodyne 1:213	none 25 min		(3) (5)	THE	(3)	0	(2)	0	(3)	2F 4F	(2)	TNC	(3)	2P 1P/2F	(2)	TNC	(3)	2P/1F 1P/2F	(3)	TNC	(3)
28. Wescodyne 1:106	none	A STATE OF THE PARTY OF THE PAR	(3)	TNC	(4)	. 0	(2)	0	(2)	1P/2F	(3)	TNC	(4)	2F	(2)	1	(3)	2F	(2)	TNC	(4)
. PHENOLICS																					
29. Boraxo	none		(4)	TNC	(4)	THE	(4)	TNC	(2)	2P	(2)	Q	(3)	2P	(2)	0	(3)	2P	(2)	1	(3)
30. Dentaseptic 1:32 31. Lysol Liquid 1:103	20 min 10 min		(4)	TNC	(4) (4)	TNC	(4)	TNC	(2) (2)	2P 1P/2F	(2)	TNC	(3)	2P 2F	(2)	TNC	(3)	2F	(2)	TNC	(3)
(Pine Scent) 32. Matar 1:256	10 min	3P	(3)	TNC	(4)	TNC	(4)	TNC	(2)	2F	(2)	1	(3)	2P/1F	(3)	10	(3)	2P/1F	(3)	2	(3)
33. Multicide 1:32 34. Omni II 1:32	10 min	4P	(4)	200 64	(3)	TNC	(4) (4)	TNC	(2)	2P/1F	(3)	0	(3)	2P 2F	(2)	1 0	(3)	2P 2F	(2)	1	(3)
35. Precise 36. Sporicidin Spray	10 min	2P/1F	(3)	90	(4)	TNC	(2)	TNC	(2)	2P 2P	(2)	0	(2)	2P 2P	(2)	0	(3)	2F 2P	(2)	58 0	(3)
30. Sporicium Spray	10 11111		(4)		(4)	1	(4)		12/		(2)		(0)		(2)		101		157		7-7
AMMONIUM																					
COMPOUNDS 37. Basic-G 1:256	none	4F	(4)	TNC	(3)	TNC	(4)	TNC	(2)	1P/2F	(3)	0	(3)	2P	(2)	0	(3)	2P	(2)	TNC	(3)
38. Hi-Tor 1:256 39. Vitawipe	none	5F 	(5)	THE	(3) (4)	TNC	(4)	TNC	(2)	1P/2F	(3)	0	(3)	2P —	(2)	1 0	(3)	2P/1F	(3)	57	(4) (3)
II. WATER CONTROLS	none		-	TNC	(50)		-		-	_	-	TNC	(10)		-	TNC	(10)		_	TNC	(10)

[†] New CoeSpray Pump has different formulation than CoeSpray. Sterall Spray is "tamed" 0.25% glutaraldehyde & is different from Sterall instrument soak.

^{*} P/F Pass/Fail. For M.bovis, Pass was designated when the disinfectant produced a 6 log₁₀ reduction in the time claimed by the company, or in 10 min, if a time was not claimed. For the other 3 bacteria, Pass results were designated when no more than 1 out of the 60 tubes showed organism growth.

[§] Cfu "Colony forming units" which denotes number of test bacteria surviving after treatment with disinfectant.

^{§§} Pfu "Plaque forming units" which denotes number of test virus surviving after treatment with disinfectants.

Not tested. Technical problems, discontinuation of product production, or inability to secure adequate quantity of product were reasons for not testing.

TNC "Too numerous to count." Virus TNC was ►200 plaque forming units. Bacteria TNC was ►500 colony forming units.

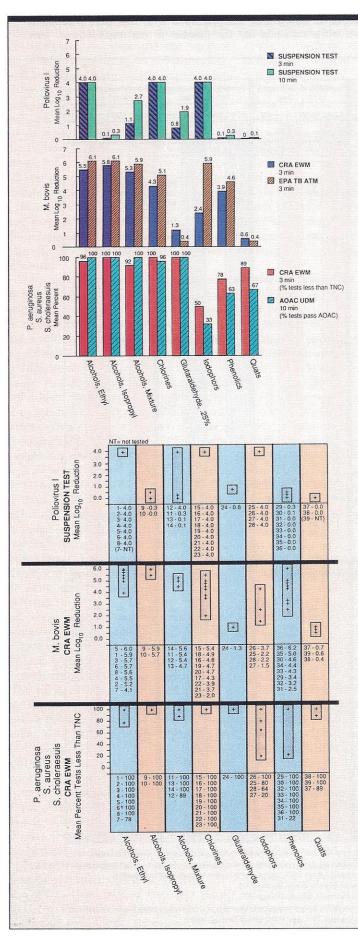


Fig 2 Comparison of results obtained with the different test methods using 3- and 10-minute contact times against the five test organisms in the absence of bioburden. Best performance is indicated by longest

Fig 3 m Individual performance of the different brands of disinfectants within the categories summarized in Figure 2. The cross is a data point that can represent one or more disinfectants, if several products performed equally. Most effective performance of the category is indicated by a single cross toward the top of a column. Variability within a category is indicated by the length of the hox enclosing several crosses. At the bottom of each column, numbers to the left of the hyphen correspond to the numbers of brand names in Table 2. In the top and middle sections, the numbers to the right of the hyphen report log10 reductions of viable organisms. In the bottom section, numbers to the right of the hyphen report the percentage of tests in which disinfectants successfully killed the three EPA-specified bacteria.

was added. Thirty minutes later, the suspension was filtered and subcultured on Mycobacterium 7H11 agar. Efficacy of the neutralizers used in TSA was evaluated by wiping CRA EWM trays with disinfectant, waiting 3 minutes, then filling the trays with TSA plus neutralizers containing about 100 CFU.

Reproducibility of the wipe test

To determine reproducibility of the mean organism challenges applied to CRA EWM test trays both without and with blood, results from water control trays were examined. Reproducibility of CRA EWM test procedures was examined when two different technicians performed the test with *M bovis* mixed 50:50 vol/vol with human whole blood; diluted Clorox (1:5) was the disinfectant. Tests were performed in parallel, and the technicians alternated tray treatment to correct as much as possible for time.

Results

Comparison of data from four different test methods

Figure 2 includes the data from all tests performed in the absence of bioburden. Disinfectants are grouped by main active ingredient to condense the data to facilitate comparison of results obtained with the four different test methods (AOAC UDM, EPA TB ATM, CRA EWM, and suspension tests). The data show a close correlation of results obtained with the EPA-specified test methods (EPA TB ATM and AOAC UDM) compared with the wipe test method (CRA EWM). Significant differences were evident only with iodophors tested against M bovis. With this particular disinfectant-test organism combination, the suspension test method (EPA TB ATM) was significantly more permissive than the surface wipe test method (CRA EWM).

Antimicrobial activity in the absence of bioburden

Table 2 lists the detailed data by CFU and PFU under each of the five test organisms for all 39 disinfectants tested in the absence of bioburden. When these data were combined under the primary active ingredient of each disinfectant (Fig 2), it became apparent that overall, in the absence of bioburden, ethyl alcohols and chlorines provided best

inactivation of all five test organisms, regardless of the test method or contact time used. Iodophors had intermediate activity. Although they performed well against the virus, they failed to kill *M bovis* dried on plastic laminate surfaces, and they had low activity against the three EPA-specified bacteria. Isopropyl alcohol, alcohol mixtures, dilute glutaraldehyde, phenolics, and quaternary ammonium compounds all failed to inactivate poliovirus, regardless of the contact time used. The glutaraldehyde and quaternary ammonium compounds also failed to kill the TB organism.

These data also showed: (1) disinfectants could kill the three EPA-specified organisms using the 10-minute EPA specified test (AOAC UDM) and not inactivate resistant organisms with clinical significance such as TB (dilute glutaraldehyde and quaternary ammonium compounds) and poliovirus (iso-

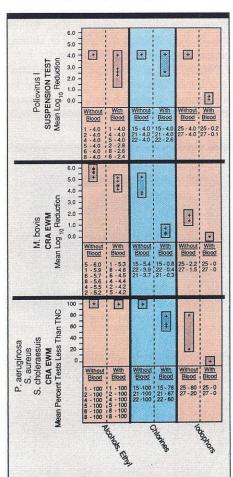


Fig 4 • Ranking by antimicrobial activity of each disinfectant within its group in the presence of human whole blood. Best performance is indicated by crosses toward the top of the graph and minimal spread of the crosses enclosed within the box. This graph is read in the same way as Figure 3.

Table 3 ■ Colony and plaque counts from tests of 11 disinfectants using the CRA EWM for the bacteria and a suspension test for the virus with 3-minute contact times in the presence of human whole blood (50% blood with bacteria and 10% with virus). Low numbers indicate good antimicrobial activity.

Disinfectants	M. b	ovis	Pollov	irus I	P. aeru	ginosa	S. chole	raesuls	S. au	reus
	Without Blood	With Blood								
ETHYL ALCOHOLS		51	0	a	0	0	0	0	0	4
Citrace	1 53	243	0	TNC	0	0	0	0	1	12
CoeSpray Ethyl, 70% v/v (denatured)	7	218	0	o o	Ö	Ö	Ö	0	0	4
Lysol sprays (Fresh Scent, Light Scent, Regular Scent, Professional)	2	48	0	0	0	0	0	0	1	2
Presept Liquid	2	164	0	TNC	0	7	. 0	1	0	17
ProCide ES	6	18	0	TNC	0	Q	0	8	0	0
CHLORINES Clorox 1:5	3	TNC	0	۵	0	94	1	0	0	TNC
Dispatch Exspor	302 TNC	TNC	0 0 0	TNC	0	63	0	0 5	0	TNC
IODOPHORS Blocide 1:213	TNC	TNC	0	TNC	0	TNC	1	TNC	TNC	TNC
Wescodyne 1:213	TNC	TNC	0	TNC	TNC	TNC	TNC	TNC	TNC	THE

TNC "Too numerous to count." Virus TNC was ►200 plaque forming units. Bacteria TNC was ►500 colony forming units.

propyl alcohol, isopropyl-ethyl alcohol mixtures, dilute glutaraldehyde, phenolics, and quaternary ammonium compounds); (2) disinfectants that killed the TB organism did not always inactivate poliovirus (isopropyl alcohols, isopropylethyl alcohol mixtures, and phenolics); (3) poliovirus was resistant to inactivation by several types of disinfectants regardless of contact time (isopropyl alcohol, dilute glutaraldehyde, phenolics, and quaternary ammonium compounds).

Figure 3 shows the performance of each of the 39 disinfectants that were included in the means reported in Figure 2. The data range within each of the eight disinfectant categories is also apparent.

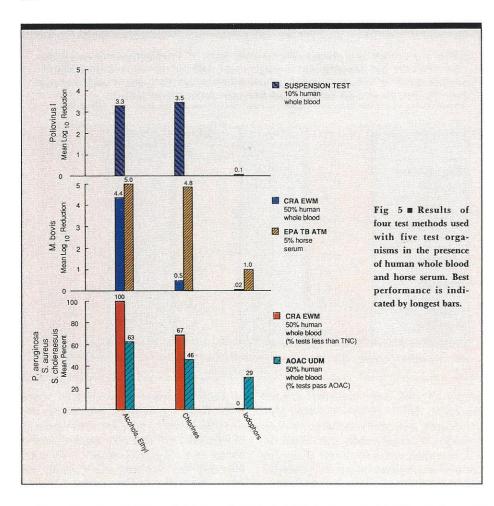
Antimicrobial activity in the presence of bioburden

Table 3 shows results of CRA EWM and suspension tests performed on the 11 disinfectants selected for testing in the presence of bioburden. These data illustrate the adverse effect of whole blood on disinfectant antimicrobial activity. Tests with eight of the 11 disinfectants produced TNC counts when blood was added to the cultures. Only Citrace, Lysol sprays, and 70% vol/vol denatured ethyl alcohol had consistently high antimicrobial activity across all five test organisms—both in the absence and presence of bioburden. Figure 4 gives a graphic representation of the data in Table 3.

In Figure 5, the 11 disinfectants listed

in Table 3 have been grouped by active ingredient to display effects of the different types and concentrations of bioburden (10% and 50% human whole blood and 5% horse serum) used with four different test methods. Overall, the ethyl alcohol category performed best regardless of test method, type or concentration of bioburden, or test organism used. The chlorine category showed high activity against poliovirus, but activity against M bovis and S aureus was dependent on type and concentration of bioburden used. Whole blood (50%) caused a significant decrease in antimicrobial activity of chlorines, whereas horse serum (5%) did not interfere with antimicrobial activity. Both brands of iodophor had very low activity across all five test organisms in the presence of all types of bioburden. Figure 6 shows the appearance of CRA EWM trays and the EPA TB ATM filters after treatment with Lysol sprays, and Biocide and Wescodyne iodophors.

Figure 7 shows results of tests performed with and without 7.0 mol/L urea, which was used to dissociate aggregates formed when ethyl alcohol products interacted with whole blood bioburden. Separate assays were performed both with the aggregates intact and after dissociation by urea. The goal was to determine if log₁₀ reductions were caused by disinfectant kill or entrapment of viable organisms within the aggregates. The data in Figure 7 indicate entrapment of viable organisms was not generally



a problem. Overall, \log_{10} reductions after urea treatment were equal to or higher than tests not including urea, indicating that these ethyl alcohol disinfectants penetrated the whole blood and inactivated the organisms within.

Antimicrobial activity related to contact time

Figure 8 shows effects of 3-minute versus 10-minute disinfectant contact times in the absence and presence of human whole blood. Generally, increasing contact time made little or no difference in the antimicrobial activity of the four disinfectants tested. Both Biocide and Wescodyne iodophors had almost no disinfectant activity in the presence of blood, even when 10-minute contact times were used. On the other hand, Citrace and Lysol sprays produced greater than 3 log₁₀ reduction for all five test organisms both at 3- and 10-minute contact times and in the absence and presence of whole blood. Therefore, Citrace and Lysol sprays were selected for testing at shorter contact times of 2 and 1 minutes.

Figure 9 shows the rapid antimicrobial activity of Citrace and Lysol sprays. In the absence of whole blood, they produced $\geq 4 \log_{10}$ reduction of all three test

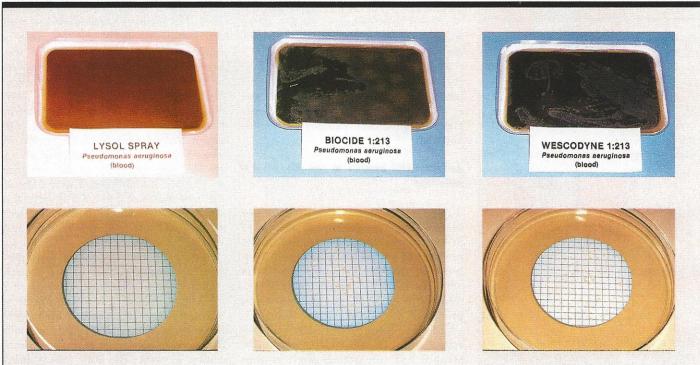


Fig 6 Top row, appearance of CRA EWM test trays contaminated with *P aeruginosa* and human whole blood after treatment with Lysol sprays and Biocide and Wescodyne iodophors. Bottom row, appearance of filters used for tests with *M bovis* and human whole blood after treatment with Lysol sprays and Biocide and Wescodyne iodophors.

organisms in 1 minute. With whole blood present, both disinfectants produced ≥ 2.8 \log_{10} reduction in 1 minute. With increased contact time, a general increase in kill was achieved.

Antimicrobial activity related to wipe material

Figure 10 shows results from tests of the hypothesis that cotton in gauze sponges used to wipe surfaces interferes with the antimicrobial activity of iodophors. These data show that iodophor antimicrobial activity was inhibited by paper towels, but cotton and rayon/polyester had no adverse effect at a 3-minute contact time.

Tests for chemical interference and neutralizer efficacy

Table 4 shows results of work performed to test for chemical interference of residuals from ETO sterilization and/or materials used to construct trays used in the CRA EWM. Colony counts of the three test organisms showed inhibition of less than 6 CFU compared with control counts, which indicates that no toxic

effects resulted from either variable.

Table 5 reports on efficacy of the neutralizers used in the CRA EWM procedure. The data show adequate neutralization of all products except quaternary ammonium compounds. Although residual activity was present to a small degree with quaternary ammonium compounds, it was not considered a problem because even with this advantage, these products failed to inactivate *M bovis* in the absence of bioburden.

Reproducibility of the wipe test

Reproducibility of the viable organism challenge on CRA EWM trays was demonstrated by the similarity of numbers of organisms computed from water control trays. The mean \log_{10} challenges of 52 (without blood) and 34 (with blood) TB water control trays were 6.01 \pm 0.22 and 6.57 \pm 0.15, respectively.

Table 6 shows results of reproducibility tests performed on the CRA EWM. Average log₁₀ reductions for *M bovis* achieved by two technicians using Clorox 1:5 in three test replications are shown.

These data were consistent both within and between technicians.

Discussion

The goal of this investigation was to identify environmental surface disinfectants that had broad-spectrum antimicrobial activity, rapid action, and effectiveness both in the absence and presence of bioburden. Only Citrace, Lysol sprays, and 70% vol/vol denatured ethyl alcohol met the criteria, regardless of the test method or contact time used. Chemically, the two commercial products are similar. Citrace contains 66.6% wt/ wt denatured ethyl alcohol (SDA-40-1), 0.12% ortho-phenylphenol, sodium nitrite rust inhibitors, proprietary deodorizer, and 3.5% hydrocarbon propellant (personal communication, Calvin Goeders, Caltech Industries, 1988) and Lysol sprays contain 79.0% wt/wt denatured ethyl alcohol (SDA-40-1), 0.1% ortho-phenylphenol, rust inhibitors, N-alkyl-N-ethyl morpholinium ethylsulfate deodorizer, and carbon dioxide propellant (personal communication, Joe Rubino, MS, Lehn and Fink, 1988).

Concentration of ethyl alcohol appeared to be a critical factor. Other products with formulations similar to Citrace and Lysol sprays, but containing less ethyl alcohol (CoeSpray with 53.5% wt/wt ethyl alcohol and ProCide ES with 52.8% wt/wt ethyl alcohol), failed to inactivate poliovirus in the presence of bioburden (Table 3). These findings led to special tests performed to investigate the antiviral activity of various concentrations of SDA-40-1 denatured ethyl alcohol used in Citrace and Lysol sprays in the presence of 10% whole blood. Results showed a dramatic increase in

Table 4 ■ Results of tests to assay for inhibitory effects of ethylene oxide residual on CRA EWM trays after ETO sterilization or chemical interference of materials used to construct trays used in the CRA EWM. Lack of interference is indicated by colony counts close to the control.

	S aureus	S choleraesuis	P aeruginosa
1. Control	74	122	125
2. Tests for ethylene oxide residual	68	128	133
3. Tests for chemical interference of tray components	81	119	121

Table 5 ■ Results of tests to confirm the efficacy of neutralizers used in the CRA EWM. Adequate neutralization is indicated by average colony counts close to the average water controls.

Disinfectant	M i	bovis	P aeru	ginosa	S chole	raesuis	S au	reus
category	Disinfectant	Water control						
Alcohols, ethyl	91	125	216	227	87	92	153	159
Alcohols, isopropyl	91	125	239	274	129	130	104	90
Isopropyl/ethyl alcohol mixes	ND	125	502	424	139	137	148	157
Chlorines	96	125	357	355	72	76	150	147
Glutaraldehyde	102	125	696	593	58	60	125	123
Iodophors	102	125	140	134	108	109	178	189
Phenols	104	125	366	353	119	121	138	145
Quaternary ammonium compounds	109	125	68	86	195	213	138	190

ND = No data

virucidal activity with alcohol concentrations equal to or greater than 70% wt/wt. Klein and Deforest⁵² reported similar results with poliovirus I. Also, many other investigators have reported very rapid inactivation of both viruses and bacteria with ethyl alcohol in concentrations of 70%-95%.^{32,57-65} However, clinicians and researchers can be misled about alcohol concentrations in commercial products if they do not understand the volume/volume and weight/weight designations.

Antimicrobial activity of high concentration ethyl alcohol can become unpredictable if storage conditions allow undetected volatilization, environmental use conditions cause extremely rapid evaporation, or interfering denaturing agents are used. Unfortunately, clinicians have no way to monitor these variables. Therefore, Citrace and Lysol sprays appeared better suited than ethyl alcohol alone for environmental surface disinfection in clinical settings because they are sealed in airtight cans to prevent volatilization during storage; they contain other ingredients in their formulations that delay evaporation during use; and their denaturing agents are standardized.

Currently, official agencies and others recommend use of iodophors, 42,66-71 chlorines, 5,42,66,69,72-75 and phenolics 75 for disinfection of environmental surfaces. Results from this investigation suggest further review of these recommendations. This work showed iodophors had very poor antimicrobial activity in the absence and presence of bioburden, regardless of the test method or contact time used. Horse serum and paper towel material caused iodophors to lose almost all activity. Many others have reported problems with iodophor antimicrobial activity on inanimate surfaces. 52,63,76-81 With chlorines, the potential of bioburden to diminish antimicrobial activity has been mentioned by many investigators52,63,78,82 and this problem was

further demonstrated in this study. The failure of different phenolic formulations to inactivate poliovirus using both 3-and 10-minute contact times was also demonstrated, and this problem has been noted by others. 6,63,83-85

In addition, this study confirmed previous reports citing inability of isopropyl alcohol to inactivate poliovirus, 52,86 problems with antimicrobial activity of dilute glutaraldehyde, 87 and inability of quaternary ammonium compounds to inactivate poliovirus 52,83,84 and the TB organism. 78,88

Precleaning of surfaces before disinfectant use has been stressed. 41,70,71,73,89-94 In the past, detergents have been preferred for this process. The obvious intent is to decrease proteins and other debris that interfere chemically with the antimicrobial activity of disinfectants. Although theoretically this appears sound, use of cleaners with low antimicrobial activity before disinfectant application causes cleaning personnel to touch concentrated body fluids containing potential pathogens. It ignores the fact that the wiping action can spread material from smaller concentrated areas to larger areas, and onto the wipe material and the person performing the cleaning. Now that disinfectants have been identified that penetrate and kill microbes within heavy bioburden, it seems prudent to apply these agents first to lower organism loads before human contact. Disinfectants containing high ethyl alcohol and ortho-phenylphenol can be used first to preclean and then disinfect in the following regimen: wet surface well and allow 2- to 3-minute disinfectant contact time to lower viable microbial load within debris; wipe vigorously to clean surfaces; rewet surface and allow 2- to 3-minute contact with disinfectant after cleaning. Appropriate barriers should be worn by cleaning personnel when using the suggested method.

Four clinically relevant points were

Fig 7 Results of assays for viable organisms with and without use of 7.0 mol/L urea to dissociate alcohol-treated blood aggregates. Best results are indicated when sets of bars are equal length or when hatched bars exceed the length of shaded bars.

demonstrated in this study:

—Disinfectants often have selective kill. Although clinicians have been advised that tuberculocidal products can be depended on to kill other important pathogens, 5 this is not necessarily true. In this study, for example, 70% vol/vol isopropyl alcohol and the phenolic, Sporicidin Spray, produced profound kill of the TB test organism (M bovis), but failed to inactivate a resistant nonenveloped virus (poliovirus). This questions the assumption of broadspectrum kill, based solely on any one organism.

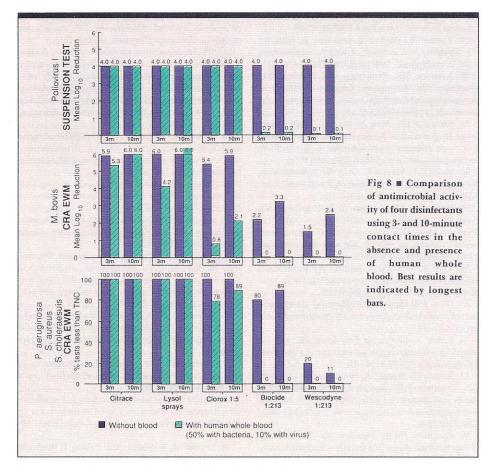
—Dilution generally decreases disinfectant activity. This effect is seen in both chlorine and iodophor categories (Table 2). Although there are reports indicating dilution increases the antimicrobial activity of iodophors, 96 this study showed activity decreased as dilution was increased from 1:106 up to 1:213. The same was true of chlorines as dilution was increased from 1:5 up to 1:20.

-Bioburden affects disinfectants adversely. The deleterious effect of bioburden on antimicrobial activity was

 Table 6 ■ Results of tests of CRA EWM reproducibility using Clorox diluted

 1:5 against M bovis mixed 50:50 vol/vol Clorox with human whole blood. Good test reproducibility is indicated by similar log₁0 reductions obtained in tests conducted by two different technicians.

Replication	Technician l	Technician 2		
A	0.61	0.61		
В	0.63	0.75		
C	0.68	0.69		
$rac{\mathbf{C}}{\mathbf{ar{X}}}$	0.64	0.68		



demonstrated repeatedly and with all disinfectants in this study to varying extents. Citrace, Lysol sprays, and 70% vol/vol denatured ethyl alcohol were affected least.

—Many commercial products have marginal activity. This point was illustrated by the fact that 28 of the 39 products in this study failed one or more test organisms even in the absence of bioburden. Only three of the 11 products tested in the presence of bioburden inactivated all five test organisms, regardless of the test method used.

Reproducibility of the AOAC Use Dilution Method has been debated for many years. In this investigation, the 60-tube version of this test was performed 230 times on 39 different products representing eight different active ingredients. When results were arranged in order of disinfectant major ingredient (Table 2), it became apparent that the AOAC UDM was reproducible when disinfectants with definite high antimicrobial activity were tested. However, when disinfectants with marginal activity were tested, variability of results increased substantially. To see this pattern, it was necessary to test several representative products from each of eight major active ingredient categories.

Conclusions

Data from this investigation indicated that optimum disinfection of environmental surfaces was highly formulation dependent. Of the 39 products tested, only three inactivated all five test organisms, regardless of test conditions. The other products showed deficiencies that contraindicate their use, in the formulation tested, as environmental surface disinfectants in clinical dental settings.

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Information about the manufacturers of the products mentioned in this article is available from the authors. Neither the authors nor the American Dental Association has any commercial interest in the products mentioned.

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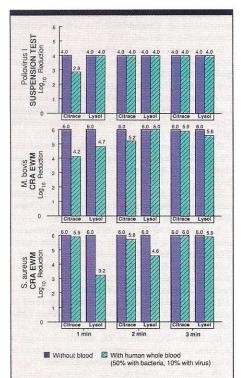


Fig 9 Mantimicrobial activity of Citrace and Lysol sprays at 1-, 2-, and 3-minute contact times in the absence and presence of human whole blood. Best performance is indicated by longest bars.

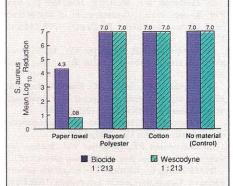


Fig 10 Tests for interference with the antimicrobial activity of Biocide and Wescodyne iodophors produced by several materials used commonly for wiping environmental surfaces. Least interference is indicated by longest bars.

section; Ms. Robinson is microbiologist; and Mr. Ploeger is virologist, microbiology section; Clinical Research Associates. Drs. Leavitt and Bodily are members of the faculty of microbiology, Brigham Young University, Provo, UT. Address requests for reprints to Dr. Christensen.

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