J. Hyg., Camb. (1978), **81**, 77
Printed in Great Britain

The relative effectiveness of commonly used disinfectants in inactivation of echovirus 11

By MURRAY DRULAK, ALFRED M. WALLBANK and INGRID LEBTAG
The Department of Medical Microbiology and Infectious Diseases, University of Manitoba Medical College, Winnipeg, Manitoba, Canada R3E 0W3

(*Received* 24 *November* 1977)

SUMMARY

Echovirus 11 in the presence of fetal calf serum was exposed to six commonly used disinfectants for times of 10, 20 and 30 s. At the end of such exposure times, skim milk neutralized disinfectant activity and residual virus was assayed using the plaque technique. The six disinfectants studied were Javex, sodium hydroxide, ethanol, Wescodyne, One Stroke Ves-Phene, and Sonacide. Although 0·25 % (w/v) sodium hydroxide and 95% (v/v) ethanol were equally virucidal and significantly more so than the other four disinfectants, causing 10^6 reduction in 20 s, they may not be practical to use in many instances. Javex at a dilution of 1/50 (1200 parts/10^6 chlorine) proved to be virucidal causing $10^{3.5}$ reduction of echovirus 11 in 30 s. Wescodyne (1/50) and undiluted Sonacide were relatively ineffective causing 10 reduction or less of echovirus 11 in 30 s. One Stroke Ves-Phene (1/50) was ineffective causing no significant inactivation in 30 s.

INTRODUCTION

Although standard procedures have been adopted for evaluating chemical bactericides and fungicides (Association of Official Agricultural Chemists, 1965) there is no standard method available for testing the virucidal ability of a disinfectant (Wright, 1970).

Bergen & Lystad (1972) conducted a study of bactericidal activity of chemical disinfectants in which it was stated that a major drawback to disinfectant testing was the inaccuracy in reporting the time bacteria were exposed to disinfectant. They attributed this to inadequate neutralization of the disinfectant activity.

In virucide testing, one approach to this problem was the use of dilution as a means of terminating the action of a disinfectant. Groupé *et al.* (1955) exposed vaccinia and influenza viruses to various potential virucides for 10 min. The virucidal activity of chemicals tested was terminated by dilution of the virus–virucide mixture in physiological saline with subsequent inoculation of such dilutions into the allantoic cavity of chick embryos. A similar test method was developed by Wright (1970) for evaluation of disinfectants against vesicular stomatitis virus. It consisted of diluting the virus–virucide mixture in phosphate buffered saline, pH 7, at the end of 5, 10 or 30 min exposure times. Virus was assayed by inoculation of egg embryos.

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

Table 1. *R3 medium*

	ml
Medium 199 (10 × Gibco*)	820
MEM amino acids (50 × Gibco)	164
MEM vitamins (100 × Gibco)	82
MEM non-essential amino acids (100 × Gibco)	82
MEM L-glutamine (100 × = 2·92 g)	82
Sodium pyruvate (100 × = 1·1 g)	100
Tryptose phosphate broth (Difco)	1000
Calf serum (Flow Labs), heat inactivated at 57 °C for 30 min	800
Distilled water	6870

* Grand Island Biological Company (Gibco) 3175 Staley Road, Grand Island, N.Y. 14072.

A test method for potential virucides analogous to the Association of Agricultural Chemists Use Dilution test for bactericides was developed (Lorenz & Jann, 1964) with modifications kept to a minimum. In this test Newcastle disease virus adsorbed to carrier rings was exposed to disinfectant for 10 min with subsequent incubation of such rings in 1 ml of nutrient broth. This broth was assayed for virus by inoculation of 0·1 ml quantities into eggs.

Gaustad, McDuff & Hatcher (1974) subsequently modified this use dilution test method using HEp-2 tissue culture cells instead of embryonated eggs as an assay system for virus, and Letheen broth (Baltimore Biological Laboratories) was substituted for nutrient broth to neutralize the disinfectant activity.

Subsequently McDuff & Gaustad (1976) reported that, owing to inconsistencies in results, this test protocol could not be adopted as a standard test for potential virucides without further modifications.

The purpose of this article is to evaluate the virucidal activity of some commonly used disinfectants on echovirus 11 using a test system that employs skim milk as a neutralizer of disinfectant activity (A. M. Wallbank, unpublished data) and short exposure times to the disinfectant. Such short exposure times were beneficial in differentiating between highly effective and ineffective disinfectants.

MATERIALS AND METHODS

R3 *medium*

R3 was prepared aseptically in 10000 ml amounts and dispensed in 500 ml quantities. It consisted of the ingredients in Table 1.

After being dispensed in 500 ml quantities, a penicillin, streptomycin and amphotericin B (Fungizone) mixture (PSF) was added to each bottle to yield a final concentration of 100 units of penicillin, 100 μg of streptomycin and 2·5 μg of Fungizone per ml of R3 medium. Before use, the pH was adjusted to 7·3 (A. M. Wallbank, unpublished).

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

Table 2. *Disinfectants and active ingredients*

Commercial name	Active ingredients	Manufacturer
Javex*	6% sodium hypochlorite	Bristol Myers Canada Ltd.
Ethanol	95% (v/v) ethanol	Commercial Alcohols.
One Stroke Ves-Phene	*O*-phenylphenol 10% *O*-benzyl-*p*-chlorophenol 8·5% *p*-tertiary amylphenol 2·0%	Vestal Labs Division of W. R. Grace Co.
Wescodyne	Polyethoxy polypropoxy polyethoxy ethanol–iodine complex 9·10% Nonylphenoxypoly (ethyleneoxy) ethanol–iodine complex 8·74% Provides 1·6% minimum titrable iodine	West Chemical Products
Sodium hydroxide	50% (w/w) NaOH	Fisher Scientific Ltd.
Sonacide	2% glutaraldehyde	Ayerst Ltd.

* Javex was purchased from local grocery stores. The chlorine content of such Javex after 6 months to 1 year of storage was 58000 to 60000 parts/10^6 as determined by the Amperometric Titration Method (Rand, Greenberg & Tarros, 1975).

Virus

The original echovirus 11 preparation was obtained from Mr Wally Stackiw of the Provincial Laboratory of Manitoba. It was designated as 2491–75. It was passaged in BGM cells (Barron, Olshevsky & Cohen 1970; Dahling, Berg & Berman 1974) 3 times before use. A pool of echovirus 11 was prepared by adsorbing an input multiplicity of 1–5 plaque forming units per cell on BGM cells in plastic 25 cm^2 Falcon flasks for 30 min at 37 °C. The infected monolayers were overlayed with 5 ml of R3 medium. When the BGM cells showed extensive cytopathic effect 24–48 h later, the tissue culture fluids were centrifuged at 1000 *g* for 10 min. The supernatant was removed and stored in 0·5 ml quantities in rubber sleeve stoppered vials at − 85 °C. Virus was thawed just before use.

The BGM cells were examined periodically for mycoplasma by the techniques of Barile (1973).

Organic material

Fetal calf serum (Flow Laboratories, 936 W. Hyde Park Blvd., Inglewood Calif. 90302) was used as added organic load in disinfectant testing. Fetal calf serum was inactivated at 57 °C for 30 min. The same batch was used in all experiments.

Disinfectants

All disinfectants tested were diluted in sterile distilled water and prepared on the day on which testing was done. The disinfectants tested are listed in Table 2.

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

Table 3. *Comparison of neutralized controls with titre for echovirus* 11

Disinfectant dilution	Trial	Titre*	Neutralized controls*	Student's *t* test results†	Dilution of disinfectant in skim milk‡
1/100 Javex	1	94, 62	80, 86	ND	1/10
	2	68, 80	74, 78	ND	
1/75 Javex	1	96, 82	88, 84	ND	1/10
	2	136, 122	132, 118	ND	
1/50 Javex	1	102, 110	136, 124	ND	1/10
	2	92, 96	100, 98	ND	
0·25% (w/v) NaOH	1	98, 104	80, 96	ND	1/10
	2	106, 110	120, 92	ND	
Undiluted Sonacide	1	158, 164	148, 162	ND	1/100
	2	92, 92	80, 94	ND	
1/200 Wescodyne	1	56, 92	64, 76	ND	1/20
	2	110, 106	100, 80	ND	
1/50 Wescodyne	1	92, 96	88, 98	ND	1/100
	2	118, 120	148, 150	ND	
1/50 Ves-Phene	1	86, 98	102, 72	ND	1/20
	2	92, 92	96, 88	ND	
95% (v/v) Ethanol	1	94, 62	128, 80	ND	1/20
	2	98, 104	96, 96	ND	

* Expressed in p.f.u./ml $\times 10^5$.
† At the 95% confidence level.
‡ 17·5% skim milk.
ND, no difference.

Temperature

Experiments were done in a laminar flow biological safety cabinet where the mean temperature was 26 °C. The temperature did not fluctuate more than 2 °C.

Minimal essential medium overlay for the plaque assay

Overlay was prepared using minimal essential medium (MEM) Auto-pow powder (Flow Laboratories). Fetal calf serum was added to the overlay such that the overlay contained 4% fetal calf serum.

Skim milk neutralizer

A 17·5% solution of skim milk (Carnation, 36·5% protein) was prepared. It was autoclaved for exactly 20 min at 121 °C in 100 ml amounts.

Plaque assay for echovirus 11

Serial tenfold dilutions of preparations on which an assay was desired (0·5 ml per plate), were absorbed on monolayers of BGM cells in 52 × 15 mm Lux tissue culture dishes for 30 min at 37 °C.

Plates were overlaid by 3 ml of a mixture of equal volumes of 1·8% Bacto-agar and MEM overlay medium. These plates were incubated in 4% CO_2 and high humidity at 37 °C for 48 h. After the 48 h period a neutral red (0·2%) agar

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

Table 4. *Disinfectants tested*

Disinfectant	Dilutions tested
Sodium hydroxide	0·25% (w/v), pH 12·5
Wescodyne	1/200, 1/50
Javex	1/100 (600 parts/10^6 chlorine), 1/75 (800 parts/10^6 chlorine), 1/50 (1200 parts/10^6 chlorine)
One Stroke Ves-Phene	1/50
Sonacide	Undiluted
Ethanol	95% (v/v)

preparation was added. The plaque count was expressed in terms of plaque forming units per ml (p.f.u./ml) of the original preparation.

Disinfectant testing

A volume of 0·4 ml of disinfectant dilution to be tested was added to a mixture consisting of 0·05 ml of fetal calf serum and 0·05 ml of echovirus 11. The reaction mixture therefore contained 10% fetal calf serum. Such 0·5 ml reaction mixtures were allowed to react for 10, 20, and 30 s. In each case at the end of the specified incubation time an appropriate amount of 17·5% skim milk (Table 3) was added to act as a neutralizer of the disinfectant.

In each experiment the effectiveness of the 17·5% skim milk neutralizer was tested by the following control, 0·05 ml of fetal calf serum and 0·4 ml of disinfectant dilution tested were added to the same amount of 17·5% skim milk as that used in the neutralization of 10, 20, and 30 s reaction samples. A volume of 0·05 ml echovirus 11 was then added. The virus was incubated in this skim milk dilution blank for a period of time equivalent to that for which the 10, 20, and 30 s samples remained in skim milk before being assayed.

Both in the case of the neutralized 10, 20, and 30 s samples and the neutralized control, 0·5 ml was serially diluted tenfold in 4·5 ml PBS-A (PBS without Ca^{2+} and Mg^{2+}, pH 7·3) (Dulbecco & Vogt, 1954) dilution blanks, with 0·5 ml amounts of the appropriate dilutions assayed for virus.

The virus was titrated at the time of each experiment. The effectiveness of the 17·5% skim milk neutralizer was determined by comparing the titre with the neutralized control results using a one tailed Student's *t* test at the 95% confidence level.

In addition to the neutralized control, another control was set up to test the ability of skim milk to neutralize the activity of the disinfectant. The main emphasis in this control, termed the cell susceptibility test control, was to determine whether or not the disinfectant was sufficiently neutralized and that toxicity of the disinfectant did not affect the ability of the cell line used in the plaque assay to be infected by the virus.

In this control, virus of known titre was diluted to the dilution preceding the one yielding between 30 p.f.u./0·5 ml and 300 p.f.u./0·5 ml. Then 0·5 ml of this

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

Table 5. *Analysis of regression results for echovirus* 11 (log_{10} *p.f.u./ml*) *versus square root minutes exposure time*

Disinfectant	Trial	r^2*	D.F.†	F
Undiluted Sonacide	1	0·806	1, 20	81·497
	2	0·869	1, 20	132·80
0·25 (w/v) NaOH	1	N.D.	N.D.	N.D.‡
	2	N.D.	N.D.	N.D.
1/50 Ves-Phene	1	N.D.	N.D.	N.D.§
	2	N.D.	N.D.	N.D.
1/200 Wescodyne	1	0·912	1, 17	176·391
	2	0·905	1, 19	181·579
1/50 Wescodyne	1	0·976	1, 20	805·422
	2	0·885	1, 20	154·230
1/100 Javex	1	0·952	1, 20	393·883
	2	0·965	1, 26	710·601
1/75 Javex	1	0·930	1, 17	224·878
	2	0·954	1, 18	373·154
1/50 Javex	1	0·966	1, 19	4259·250
	2	0·987	1, 20	1489·046
95% (v/v Ethanol	1	N.D.	N.D.	N.D.‡
	2	N.D.	N.D.	N.D.

* Correlation coefficient squared.

† Degrees of freedom for the F statistic.

‡ N.D., not done due to a rapid viral inactivation yielding a relationship of less than 3 numerical points.

N.D., not done due to no significant viral inactivation occurring.

preceding dilution was added to 4·5 ml of a mixture of disinfectant–skim milk identical in proportion to that found in the neutralized virus–disinfectant mixture.

Virus was assayed using the plaque assay.

The titre of the virus was compared with the cell susceptibility control test results, using the Student's t test at the 95% confidence level.

RESULTS AND DISCUSSION

Echovirus 11 was exposed to dilutions of disinfectants listed in Table 4. In virus–disinfectant interactions residual virus versus exposure time was plotted as log $_{10}$ p.f.u. versus square root minutes exposure time (Figs. 1–5). Each plotted point represents the mean of 4–8 replicate observations. Analysis of regression was done in all cases with the exception of those in which no significant decrease in virus was noted upon exposure to the disinfectant (e.g. Fig. 1) and in those cases in which disinfectant inactivation of the virus was so rapid, that less than 3 numerical values were obtained (e.g. Fig. 2). Results in Table 5 consisted of an F statistic which was the ratio of (mean square regression)/(mean square deviation) and the correlation coefficient squared value (r^2). r^2 is the proportion of the variation of the dependent variable (residual virus) explained by the regression equation (Steel & Torrie, 1960).

In all instances the F values exceeded 50 and therefore are highly significant at the 95% confidence level indicating linearity. In addition all r^2 values were over 0·8.

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

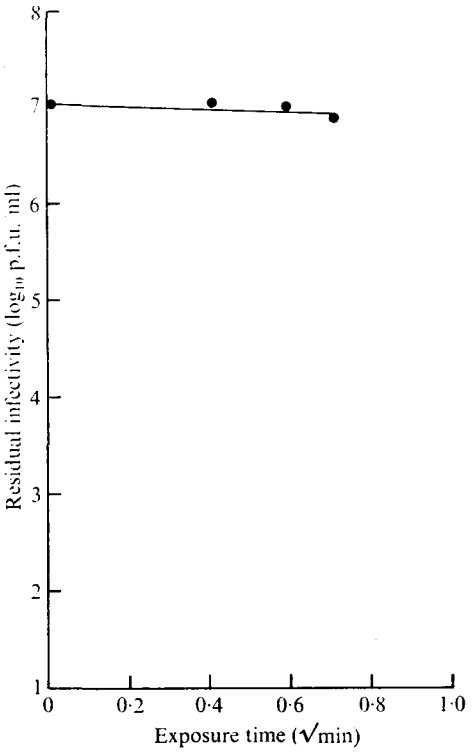

Fig. 1

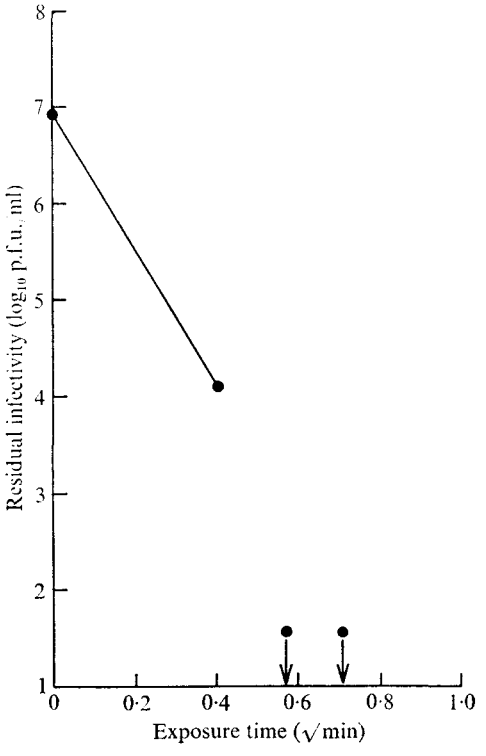

Fig. 2

Fig. 1. Echovirus 11 in fetal calf serum exposed to 1/50 Ves-Phene. Residual infectivity ($\log_{10}$ p.f.u./ml) versus square root minutes exposure time.

Fig. 2. Echovirus 11 in fetal calf serum exposed to 0·25% (w/v) sodium hydroxide Residual infectivity ($\log_{10}$ p.f.u./ml) versus square root minutes exposure time.

The lines (Figs. 1–5) are based on the calculated values for the linear regression which was fitted around the actual mean data values.

Two experimental trials for each viral inactivation were done. In these instances where data were conducive to statistical analysis, the reproducibility of the inactivation relationship was determined by comparing the slopes of the different experimental trials statistically at the 95% confidence level. In all instances in Table 6 the slopes of the two experimental trials were not significantly different at the 95% confidence level with the exception of echovirus 11 in fetal calf serum treated with 1–100 (600 parts/10^6 chlorine) Javex. In this case the slopes were significantly different at the 95% confidence level but not at the 99% confidence level.

In instances where analysis of regression was done, the relative efficiency with which each disinfectant inactivated echovirus 11 was determined by comparison of slopes at the 95% confidence level. In comparisons where one or both of the residual virus versus exposure time relationship was not conducive to analysis of regression, non-statistical comparisons had to be made. For example, in Fig. 2, 0·25% (w/v) NaOH inactivated echovirus 11 by 10^6 in 20 s whereas 1/50

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

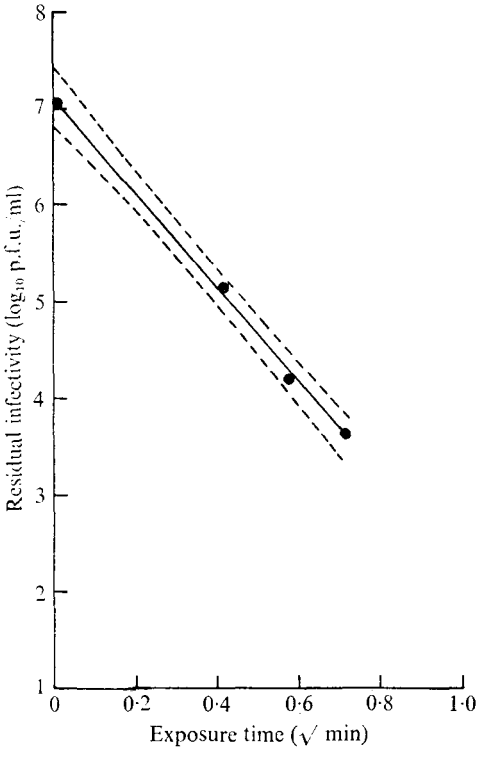

Fig. 3

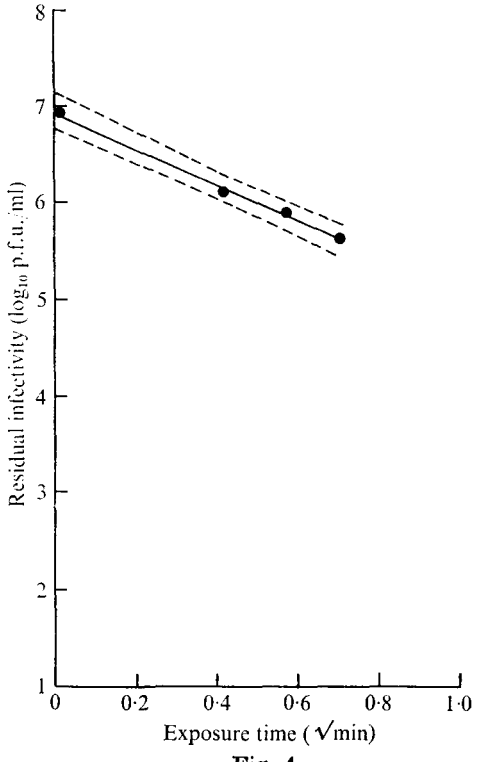

Fig. 4

Fig. 3. Echovirus 11 in fetal calf serum exposed to 1/50 (1200 parts/10^6 chlorine) Javex. Residual infectivity ($\log_{10}$ p.f.u./ml) versus square root minutes exposure time.

Fig. 4. Echovirus 11 in fetal calf serum exposed to 1/50 Wescodyne. Residual infectivity ($\log_{10}$ p.f.u./ml) versus square root minutes exposure time.

(1200 parts/10^6 Javex (Fig. 3) inactivated echovirus 11 by 10^3 in 20 s, therefore 0·25% (w/v) NaOH was a more effective virucide than 1/50 (1200 parts/10^6) Javex.

Conclusions of statistical and non-statistical comparisons are listed in Table 7.

Ethanol (95% v/v) and sodium hydroxide (0·25% w/v) were the two most effective virucides with reference to echovirus 11. Both of these agents were equally effective since they both inactivated 10^6 virus particles in 20 s, which none of the other disinfectants could do (e.g. Fig. 2).

The term 95% (v/v) ethanol is a misleading one, since the test system consisted of 4 parts of disinfectant to 0·5 part virus and 0·5 part serum. This meant the 95% (v/v) ethanol added was diluted to $4/5 \times 95\% = 76\%$ (v/v) ethanol.

Although 95% (v/v) ethanol was effective in this test system, ethanol must be used with caution since it cannot be diluted for use the way many other disinfectants can. Therefore, ethanol is unreliable to use unless in a large volume in comparison with the volume of virus-contaminated fluid, to which ethanol is applied.

Sodium hydroxide (0·25% w/v) had a pH of 12·5 making it too caustic to use as a virucide in many situations, except perhaps in veterinary medicine.

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

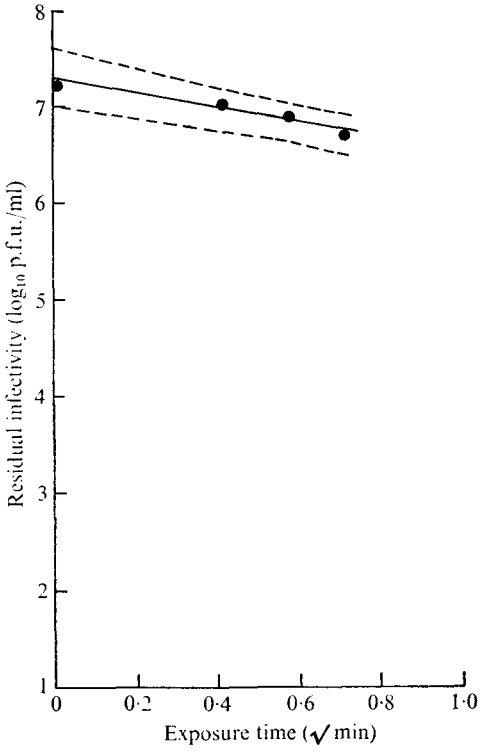

Fig. 5. Echovirus 11 in fetal calf serum exposed to undiluted Sonacide. Residual infectivity ($\log_{10}$ p.f.u./ml) versus square root minutes exposure time.

A dilution of 1/50 (1200 ppm) Javex was the third most effective virucide for echovirus 11 inactivation (Fig. 3) with $10^{3.5}$ reduction occurring in 30 s. Javex was not only an effective virucide but is also more practical to use than either ethanol or sodium hydroxide.

A 1/50 dilution of Wescodyne was ranked fourth in effectiveness for echovirus 11 inactivation with 10^{1} reduction occurring in 30 s (Fig. 4). The recommended use dilution of Wescodyne is approximately 1/200 which for an exposure time of 5 min was quoted as being virucidal for poliovirus in the literature of the manufacturer (West Chemical Products Inc.). In a 30 s exposure time at such a dilution echovirus 11 was also reduced by 10^{1}. This indicated that Wescodyne was ineffective in relatively short exposure times, since Wescodyne used at 4 times the recommended strength did not increase in virucidal ability.

Undiluted Sonacide was ranked as the second least effective disinfectant of the six considered. Figure 5 shows less than a 10^{1} reduction of echovirus 11 occurred in 30 s with undiluted Sonacide. The manufacturer recommends use of undiluted Sonacide for an exposure time of 10 min.

A dilution of 1/50 One Stroke Ves-Phene was totally ineffective in inactivating echovirus 11 in 30 s (Fig. 1), thus making One Stroke Ves-Phene the least effective of the six disinfectants tested. The recommended use dilution of One Stroke Ves-Phene is 1/256.

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

Table 6. *Reproducibility of the $\log_{10}$ p.f.u./ml versus square root minutes relationship for echovirus* 11*

Disinfectant	Trial	N†	Square root (min) Mean	Square root (min) S.D.	$\log_{10}$ p.f.u. per ml Mean	$\log_{10}$ p.f.u. per ml S.D.‡	Slope	D.F.§	F
95% Ethanol	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.‖
	2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		
0·25% NaOH	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.‖
	2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		
1/100 Javex	1	22	0·462	0·249	2·132	0·464	−1·815	1, 46	5·220¶
	2	28	0·484	0·231	1·840	0·492	−2·092		
1/75 Javex	1	19	0·446	0·261	1·797	0·844	−3·121	1, 35	3·018
	2	20	0·459	0·261	1·946	0·720	−2·698		
1/50 Javex	1	21	0·464	0·255	2·829	1·254	−4·904	1, 39	3·013
	2	22	0·462	0·249	2·621	1·291	−5·143		
1/50 Wescodyne	1	22	0·462	0·249	2·081	0·478	−1·895	1, 40	2·230
	2	22	0·462	0·249	2·220	0·442	−1·667		
1/200 Wescodyne	1	19	0·446	0·261	2·200	0·411	−1·506	1, 36	0·676
	2	21	0·450	0·249	2·189	0·431	−1·645		
Undiluted Sonacide	1	22	0·462	0·249	1·898	0·222	−0·799	1, 40	0·035
	2	22	0·462	0·249	1·635	0·208	−0·778		
1/50 Ves-Phene	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.‖
	2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		

* Determined by comparison of slopes at the 95% confidence level.
† Total number of experimental observations.
‡ Standard deviation.
§ Degrees of freedom.
‖ Not done, since data was not conducive to analysis of regression.
¶ Significant at the 95% but not the 99% confidence level.

Table 7. *Relative effectiveness of the six disinfectants in inactivation of echovirus* 11

	NaOH 0·25%	Wesco-dyne 1/200	Wesco-dyne 1/50	Javex 1/000	Javex 1/75	Javex 1/50	Ves-phene 1/50	Ethanol 95%
Wescodyne 1/200	−							
Wescodyne 1/50	−	ϕ						
Javex 1/100	−	+	ϕ					
Javex 1/75	−	+	+	+				
Javex 1/50	−	+	+	+	+			
Ves-phene 1/50	−	−	−	−	−	−		
Ethanol 95%	ϕ	+	+	+	+	+	+	
Sonacide 1/1	−	−	−	−	−	−	+	−

− = is less effective than; + = is more effective than; ϕ = is not different from.

Table 3 lists the neutralized controls and the titres for each inactivation relationship as well as the dilution with 17·5% skim milk used for the neutralization. A Student's t test comparison at the 95% confidence level between the titre and neutralized control in each instance indicated the neutralized control values were not significantly less than the titre. In addition, a Student's t test comparison

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press

between the titre and the cell susceptibility test control at the 95 % level showed in each case that the cell susceptibility test control was not significantly less than the titre. On this basis it can be concluded that 17·5% skim milk was an effective neutralizer of the activity of the diverse group of disinfectants tested.

We thank Dr N. Stephens for helpful suggestions with the statistics and Mr R. Rollwagon for additions to the University of Manitoba Health Science On-Line Computer package which made our statistical analysis more convenient to carry out. This investigation was suppported by grant 607-1008-28 from Health and Welfare Canada.

REFERENCES

Association of Official Agricultural Chemists (1965). *Official Methods of Analysis* 10th ed. Association of Official Agricultural Chemists, Washington, D.C.

Barile, M. F. (1973). *Mycoplasmal Contamination of Cell Cultures: Mycoplasma–virus-Cell Culture Interactions Contamination in Tissue Culture*, ch. 6. New York and London: Academic Press Inc.

Barron, A. L. C., Olshevsky, C. & Cohen, M. M. (1970). Characteristics of the BGM line of cells from African green monkey kidney. *Archiv für die gesamte Virusforschung* **32**, 389–92.

Bergen, T. & Lystad, A. (1972). Evaluation of disinfectant inactivators. *Acta pathologica et microbiologica scandinavica*, Section B, **80**, 507–10.

Dahling, D. R., Berg, G. & Berman, D. (1974). BGM; A continuous cell line more sensitive than primary Rhesus and African green kidney cells for the recovery of viruses from water. *Health Laboratory Science* **11**, 275–82.

Dulbecco, R. & Vogt, M. (1954). Plaque formation and isolation of pure lines with poliomyelitis viruses. *Journal of Experimental Medicine* **99**, 167–82.

Gaustad, J. W., McDuff, C. R. & Hatcher, H. J. (1974). Test method for the evaluation of virucidal efficacy of three common liquid surface disinfectants on a simulated environmental surface. *Applied Microbiology* **28**, 748–52.

Groupé, V., Engle, C. G., Gaffney, P. E. & Manaker, R. A. (1955). Virucidal activity of representative antiinfective agents against influenza A and vaccinia viruses. *Applied Microbiology* **3**, 333–6.

Lorenz, D. E. & Jann, G. J. (1964). Use-dilution test and Newcastle disease virus. *Applied Microbiology* **12**, 24–6.

McDuff, C. R. & Gaustad, J. W. (1976). Test method for determination of virucidal efficacy of liquid surface disinfectants. *Journal of the Association of Official Analytical Chemists* **59**, 1150–55.

Rand, M. C., Greenberg, A. E. & Tarros, M. J. (1975). *Standard Methods for the Examination of Water and Wastewater*, 14th edn, pp. 322–5. American Health Association, Washington, D.C.

Steel, R. & Torrie, J. (1960). *Principles and Procedures of Statistics*, ch. 10, p. 187. New York: McGraw and Hill Co.

West Chemical Products Inc. *Wescodyne. Detergent-Germicide for hospital use containing 'Tamed Iodine'*, West Chemical Products Inc. 42–16 West Street, Long Island City, N.Y. 11101.

Wright, H. S. (1970). Test method for determining the virucidal activity of disinfectants against vesicular stomatitis virus. *Applied Microbiology* **19**, 92–5.

https://doi.org/10.1017/S002217240005378X Published online by Cambridge University Press