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Inactivation of HIV-1 by chemical disinfectants: sodium hypochlorite

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SUMMARY

The efficacy of sodium hypochlorite was assessed against human immunodeficiency virus type 1 suspended in low (8% v/v) or high (80% v/v) concentrations of serum or in a high (80%) concentration of blood. In the presence of 8% serum, 100 p.p.m. available chlorine in the disinfectant test mixture inactivated 3·75 log $TCID_{50}$ HIV/ml within 30 s. When the test mixture contained 80% serum, 500 p.p.m. available chlorine inactivated more than 4 log $TCID_{50}$ HIV/ml in 1–2 min. Lower concentrations of available chlorine were unable to inactivate the virus completely. In the presence of 80% blood, 1000 p.p.m. available chlorine in the disinfectant test mixture was unable to inactivate 3·75 log $TCID_{50}$ HIV/ml, although 2500 p.p.m. available chlorine was able to inactivate at least 1·5 log $TCID_{50}$ HIV/ml. In all test mixtures, the chlorine rapidly became combined and thus less active. Our results emphasise the importance of cleaning prior to disinfection with sodium hypochlorite since it may prove to be ineffective in the presence of high levels of organic matter. In cases where prior cleaning is impossible, care must be taken to use the higher recommended concentration (a minimum of 10000 p.p.m. available chlorine).

INTRODUCTION

Disinfectants containing hypochlorites are recommended for a wide variety of uses including the decontamination of infected blood and body fluid spillages [1–3]. Nevertheless, they suffer from a number of disadvantages including corrosive properties, deterioration when exposed to heat and light and rapid neutralization of their microbicidal activity by proteinaceous material.

The oxidizing (chlorinating) capacity of a hypochlorite solution is expressed as the level of available chlorine in percent or parts per million (p.p.m.). One percent is equivalent to 10000 parts per million. Currently, the World Health Organisation (WHO) recommends the use of chlorine releasing compounds at 5000 p.p.m. available chlorine in dirty conditions (e.g. flooding contaminated surfaces prior to the removal of bulk material) for a minimum exposure time of 10 min, and between 500 and 1000 p.p.m. available chlorine for clean conditions following the

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physical removal of bulk material [3] with no minimum exposure period. Guidelines in the UK recommend 1000 p.p.m. available chlorine for contaminated surfaces and 10000 p.p.m. available chlorine for gross spillages [2], whilst the Centers for Disease Control in the US recommended 500–5000 p.p.m. available chlorine, depending upon the quantity of organic matter present [1].

The precise mechanism by which chlorine exerts its microbicidal effect is complex and poorly understood. The total available chlorine in a solution may be free or combined. Free available chlorine consists of hypochlorous acid, a strong oxidizing agent, elemental chlorine and hypochlorite ion. Combined available chlorine is formed by the reaction of sodium hypochlorite with organic matter (e.g. blood, faeces, tissues) causing the production of chloramines or *N*-chloro-compounds. It is generally considered that the greatest antimicrobial activity is caused by the oxidation of cell proteins by hypochlorous acid, although the mechanism has never been demonstrated experimentally [4–6].

Many previous studies on HIV inactivation by chlorine releasing agents have not addressed adequately of some of the important factors in disinfectant testing. These include the initial viral load, the sensitivity of the assay, the level of protein loading and the proportion of free and combined available chlorine in the disinfectant test mixtures. In the absence of a suitable neutralizer of the disinfectant, and without an assessment of the toxic effect of the disinfectant or disinfectant/neutralizer combination on the cell line used to detect residual infectious virus, there remains the possibility that the effectiveness of disinfectant activity may be exaggerated.

In this study, virus was suspended in a low concentration of serum to assess the activity of the disinfectant when minimal organic matter is present and in a high concentration of serum or blood to simulate 'worst-case' *in vivo* conditions.

METHODS

Virus stock

The RF strain of HIV-1 was maintained by continuous culture in MT-4 cells suspended in complete medium (RPMI 1640 supplemented with 10% heat inactivated foetal calf serum (FCS), 5 mmol l^{-1} HEPES and 50 mg l^{-1} gentamicin). Cell free virus (CFV) was prepared as described previously [7].

Disinfectants

Sodium hypochlorite (Merck Ltd, Lutterworth, Leicestershire) was diluted in water of standard hardness [8] to a stock concentration. This was diluted subsequently by its addition to the virus plus test medium (complete medium containing 10% FCS, FCS or blood). The concentration of disinfectant in contact with the virus in the disinfectant test mixture was described as the final concentration.

Neutralizers

Sodium thiosulphate (Merck Ltd, Lutterworth, Leicestershire) and protein (FCS; Life Technologies Ltd, Inchinan, Renfrewshire, Scotland) were assessed for their efficacy as neutralizing agents.

Virucidal suspension tests

All experiments were done at room temperature.

In serum

Stock virus or tenfold dilutions of virus (100 μl) were suspended in 800 μl complete medium (CM) or FCS. Disinfectant (100 μl) was added, mixed and left for the required test period. The concentration of serum in the test mixture was therefore 8% v/v for virus suspended in CM or 80% v/v for virus suspended in FCS. Each sample was tested in duplicate. Neutralizer was added immediately after the exposure period to prevent or reduce further action of the disinfectant. The volume of neutralizer was 1 ml when the test medium was FCS, but this was increased to 8 ml when the test medium was CM. Freshly split MT-4 cells (4 ml, 10^5–10^6 cells/ml) were diluted in CM for tests in FCS and concentrated in FCS for tests in CM to produce a final volume of 10 ml when added to the disinfectant/neutralizer mixture. The virus was allowed to absorb to the cells by mixing gently on an orbital shaker for 1 h at room temperature. The mixture was centrifuged at 130 g for 10 min, resuspended in 4 ml of CM and 1 ml of this mixture was added to each of 4 wells of 24-well tissue culture plates. The plates were incubated at 37 °C in 5% CO_2 and the cultures were examined for a cytopathic effect (CPE) every 3–4 days. Half the volume in each well was replaced with either CM (CPE negative wells) or freshly split cells (CPE positive wells). After 21 days, samples were taken for measurement of p24 antigen levels (DuPont (UK) Ltd, Stevenage, Herts.). An untreated control, where the virus did not come into contact with the disinfectant, was included in each experiment to determine loss of viral activity during the experimental procedure. The disinfectant test concentration was diluted by one tenth at the adsorption stage of the experiment and loss of virus due to residual activity during this step was determined by the inclusion of a second, 'adsorption' control. In both the controls, sodium hypochlorite was substituted by CM. Immediately prior to adding the cell suspension, the disinfectant and neutralizer were added to the adsorption control.

In blood

The protocol described had to be modified when testing in blood. Fresh human blood was collected from healthy volunteers into tubes containing the anticoagulant EDTA and mixed by inverting a minimum of six times. EDTA was chosen since this has been reported to have no effect on the recovery of virus from blood [9]. The standard suspension test protocol was followed, but the red blood cells had to be removed before the adsorption stage to avoid interference in the cytotoxicity assay and problems with the red blood cells masking the CPE in the infectivity assay. The disinfectant was added to the blood/virus mixture in a 2 ml plastic tube and left for the required time. Neutralizer (500 μl) was added, the mixture was left for 1 min and centrifuged at 13440 g in a microfuge for 15 s to pellet the red blood cells. The supernatant was added to 9 ml MT-4 cells suspended in FCS, the virus was allowed to adsorb to the cells and the procedure continued as described for FCS.

Virus infectivity was indicated by the presence of a CPE and the log $TCID_{50}$/ml was calculated by the method of Kärber [10].

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Cytotoxicity assay

Although the disinfectant was removed from each sample prior to culturing the virus, toxicity to the cells was important during the 1 h adsorption of virus onto the cells. At this stage, the disinfectant had been diluted to a tenth of the final concentration in the viral test mixture. The cytotoxicity of the disinfectant during the adsorption phase was determined prior to virucidal testing. The method used was an adaptation of a colorimetric assay for the growth and survival of cells using a yellow tetrazolium salt which is cleaved by metabolically active cells into a blue formazan product [11]. The amount of cleavage relates directly to the number of metabolically active cells present. The assay initially followed that of the standard assay for inactivation of virus in suspension, but with CM substituted for virus. The final suspension was pipetted into 16 wells of a microtitre plate (100 μl per well) and incubated overnight at 37 °C in 5% CO_2. The following day 10 μl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Chemical Company, Poole, Dorset) diluted to 5 g l^{-1} was added to each well and the plates incubated at 37 °C for 4 h. This allowed metabolism of the yellow dye by the cells, resulting in formation of blue crystals. The reaction was stopped by the addition of 100 μl acid isopropanol (0·04 M hydrochloric acid in isopropanol). Once the crystals had dissolved completely, the plates were read on a plate reader at 570 nm with a reference wavelength of 630 nm. The mean of the 16 replicates for each set of conditions was calculated and compared as a percentage of the control cells which were taken as 100% metabolic activity. Values above 90% were considered to be equivalent to the controls and a disinfectant/neutralizer concentration with a value below 90% of the metabolic activity of the control cells was taken to be cytotoxic. The disinfectant concentration in the assay is given as the concentration in the test mixture, tenfold higher than the concentration during the adsorption stage when the disinfectant/neutralizer mixture is acting on the cells.

Titration of available chlorine

Sodium arsenite method

The total available chlorine contained in the hypochlorite solution as received from the manufacturer, was titrated by the sodium arsenite method [12] 18–24 h prior to each cytotoxicity or virucidal test. The required concentrations of hypochlorite solution were prepared for use the following day and stored in the dark at 4 °C. All solutions were allowed to warm to room temperature before use.

DPD ferrous titrimetric assay

The presence of blood or serum created an indeterminate endpoint in the sodium arsenite method so we used the DPD (*N*,*N*-diethyl-*p*-phenyldiamine) ferrous titrimetric assay [13] to analyse the available chlorine in the disinfectant test mixtures. This method measures both free and combined available chlorine.

The disinfectant test mixture consisted of nine parts test medium to one part sodium hypochlorite. The available chlorine concentration was assayed either immediately or after standing times of 30 or 60 min. The effect of adding neutralizer to the test mixture (at a ratio of 0·5:1) was also examined after a 1 min contact time.

RESULTS

Cytotoxicity

Sodium thiosulphate (1%) neutralized the cytotoxic effects of 2500 p.p.m. available chlorine in 80% serum, representing 250 p.p.m. available chlorine during the adsorption stage of the protocol (Table 1), FCS (8 ml) with cells suspended additionally in neat FCS during the adsorption stage, neutralized 1000 p.p.m. available chlorine in CM, representing 100 p.p.m. available chlorine during adsorption (Table 1).

Virucidal assays

All virucidal assays were performed at or below the maximum measured non-cytotoxic disinfectant/neutralizer concentration. When CFV was tested in CM a reduction in viral titre of over 3·75 log $TCID_{50}$/ml occurred within 30 s contact time with a maximum concentration of 100 p.p.m. available chlorine in the disinfectant test mixture, representing a stock concentration of 1000 p.p.m. (Table 1). When the level of serum was increased to 80% and the maximum concentration of available chlorine was 500 p.p.m. or greater, a reduction of more than 4·0 log $TCID_{50}$/ml was achieved between 1 and 2 min (Table 2).

In marked contrast to the results in serum, in the presence of 80% blood a concentration of 1000 p.p.m. available chlorine in the disinfectant test mixture (representing a concentration of 10000 p.p.m. in the stock solution) was unable to inactivate 3·75 log $TCID_{50}$/ml of virus (Table 3). Testing in blood was problematical at concentrations of available chlorine equal to and above 1000 p.p.m. in the test mixture. Higher concentrations and longer test periods caused haemolysis, partially obscured the pelleted cells and caused difficulty in removing the supernatant. This was particularly evident for a test concentration of 2500 p.p.m. available chlorine, representing a stock concentration of 25000 p.p.m. available chlorine. The amount of virus removed in the adsorption control was low and likely to be an underestimate, although all test results were negative, indicating a minimum inactivation of 1·5 log $TCID_{50}$/ml.

In view of the problems at higher available chlorine concentrations in 80% blood, we tried 40% blood as the test medium. In 40% blood concentrations of 1000 p.p.m. available chlorine in the disinfectant test mixture appeared to inactivate virus at up to 30 min, but not at 60 min (Table 3). This apparent contradiction is probably due to inactivation of virus at or around the level of the maximum measurable drop in titre of 2·25 log $TCID_{50}$/ml.

Titration of available chlorine

We found the arsenite method of chlorine titration to be quick, accurate and reproducible for hypochlorite solutions which did not contain additional protein. However, the presence of protein created an indeterminate endpoint and so the analysis of available chlorine levels in the disinfectant reaction mixtures was by the DPD assay. A large proportion of the available chlorine rapidly became combined in the disinfectant test mixtures, independent of the amount of proteinaceous material present (Table 4). Free available chlorine could not be

Table 1. *Inactivation of cell free virus in 80% complete medium by NaOCl*

ch	ilable (p.p.m.)	Control titre ($TCID_{50}$/ml)		Log dilution factor	Maximum recovered titre	Results after treatment (min)	
St	Final	Untreated	Adsorption			Virus present	Virus abse
1	100	5·60	5·35	1·60	3·75		0·5, 1, 2, 5,
2	250	5·60	5·10	1·60	3·50		0·5, 1, 2, 5,
5	500	5·60	4·35	1·60	2·75		0·5, 1, 2, 5,
10	1000	5·60	3·10	1·60	1·50		0·5, 1, 2, 5,

Sodium hypocl e solution was added to cell free virus suspended in complete medium (CM). The neutralizer was 8 ml FCS and c re suspended in foetal calf seru ntreated: untreated control (titre of virus in the absence of disinfectant or neutralizer). Adsorption: adsorpti ntrol (titre of *virus after the*] *ice of disinfectant during the adsorption stage*). *Log dilution factor: dilution factor for virus* (log TCII l) during the experimental pr e. Maximum recovered titre: maximum measurable titre of virus (adsorption control minus log dilution fa

Table 2. *Inactivation of cell free virus in 80% serum by sodium hypochlorite*

ch	ilable (p.p.m.)	Control titre ($TCID_{50}$/ml)		Log dilution factor	Maximum recovered titre	Results after treatment (min)	
St	Final	Untreated	Adsorption			Virus present	Virus abse
1	100	6·6	6·89	1·60	5·25	1, 2, 5, 10, 15, 30	
2	250	6·6	6·10	1·60	4·50	1, 2, 15	5, 10, 30
5	500	5·35	6·10	1·60	4·50		1, 2, 5, 10, 15,
10	1000	5·35	5·60	1·60	4·00	1	2, 5, 10, 15, 30
25	2500	7·10	5·60	1·60	4·00		1, 2, 5, 10, 15,

Sodium hypoc e was added to virus in complete medium. The neutralizer was 1 ml 1% sodium thiosulphate. For other abbre ns see legend to Table 1.

Table 3. *Inactivation of cell free virus in blood by sodium hypochlorite*

ch	ilable (p.p.m.)	Control titre ($TCID_{50}$/ml)		Log dilution factor	Maximum recovered titre	Results after treatment (min)	
St	Final	Untreated	Adsorption			Virus present	Virus abse
1	100	6·35	5·85	1·60	4·25	1, 2, 5, 10, 15, 30	
2	250	6·35	6·10	1·60	4·50	1, 2, 5, 10, 15, 30	
5	500	6·35	6·10	1·60	4·50	1, 2, 5, 10, 15, 30	
10	1000	5·10	5·35	1·60	3·75	5, 10, 15, 30, 60†	
25	2500	5·10	3·10	1·60	1·50		5, 10, 15, 30, 6(
2	1000	4·60	3·85	1·60	2·25	60	2, 5, 10, 15, 30

Sodium hypocl · was added to virus suspended in 80% blood or * 40% blood. In all cases the neutralizer was 0·5 ml FCS, suspended in FCS. † All samp ·re haemolysed by the disinfectant. For other abbreviations see legend to Table 1.

Table 4. *Estimation of available chlorine levels in disinfectant test mixtures*

Test medium	Maximum available Cl_2 (p.p.m.) in: Disinfectant test mixture	Disinfectant neutralizer mixture	Standing time (min) after addition of: NaOCl	FCS*	% available Cl_2 remaining: Free	Combined	Total
CM	100	66·7	0	1	0	24·7	24·7
			30	1	0	10·3	10·3
			60	1	0	8·5	8·5
FCS	100	100	0	NN	0	31·7	31·7
			30	NN	0	12·3	12·3
			60	NN	0	6·5	6·5
Blood	100	100	0	NN	0	45·8	45·8
			30	NN	0	27·1	27·1
		66·7	30	1	0	3·8	3·8
FCS	250	250	0	NN	< 1·0†	62·7	63·7
			30	NN	0	38·7	38·7
			60	NN	0	33·0	33·0
Blood	250	250	0	NN	5·6	66·1	71·7
			30	NN	0	45·0	45·0
		166·7	30	1	0	24·0	24·0

The level of free and combined available chlorine was measured in different disinfectant test mixtures (virus, represented by CM, test medium plus disinfectant). max. av. Cl_2: maximum available chlorine. TM: test medium. DTM: disinfectant test mixture (NaOCl plus viral growth medium). DNM: disinfectant neutralizer mixture (DTM plus neutralizer). NN, no neutralizer. * FCS added at the ratio of 1:2 parts of disinfectant test mixture. † < 1·0 indicates development of a pink colour (see assay method), but loss of the colour was below the minimum limit of titration.

detected in any of the test mixtures containing an initial concentration of 100 p.p.m. available chlorine and was only measurable using 250 p.p.m. available chlorine when blood was used as the test medium. When foetal calf serum was added as a neutralizer, the levels of combined available chlorine decreased further. The standing time of either 30 or 60 min also reduced the levels of combined available chlorine.

DISCUSSION

The methods described in this study have attempted to address the factors so often ignored or inadequately dealt with in disinfectant testing. A high concentration of organic matter was used as the test medium to simulate worst-case conditions and prior to virucidal testing, suitable neutralizers were assessed for their ability to eliminate cytotoxicity prior to virucidal testing.

Data on the inactivation of HIV by sodium hypochlorite and sodium dichloroisocyanurate (NaDCC) are summarized in Table 5, although differences in experimental design such as the assay system used to measure presence of virus, the protein load and the form of neutralization if any, permit only limited comparisons between these studies. In addition, caution should be exercised in the interpretation of results if the available chlorine content of the hypochlorite stock solution and/or test solutions are not reported to have been quantified at the time

Table 5. *Su ry of published data on inactivation of HIV by sodium hypochlorite (NaOCl) and sodium dichlo yanurate (NaDCC)*

Concentration of available chlorin (formulation)		Contact time	Organic matter	Assay system	Neutralizer	Toxicity control	Available chlorine measured	Percentage reduction in viral titre, (% RT) or p2 antigen	Reference no.
50 p.p.m. (N	NaDCC)†	2 min	None	Infectivity	$Na_2S_2O_3$	Yes	Yes	2×10^4/ml	[8]
100 p.p.m. (N	NaDCC)†	2 min	None	Infectivity	$Na_2S_2O_3$	Yes	Yes	2×10^4/ml	[8]
2000 p.p.m. (N		1 h	None	RT	None	No	No	99% RT activi	[29]
10000 p.p.m. (N		1 min	None	p24 antigen	Dilution	Yes	No	56 ng p24	[2]
52500 p.p.m. (N		30 s	None	p24 antigen	None	Yes	No	⩾ 100 pg/ml	[28]
1000 p.p.m. (N		10 min	10% serum	Infectivity	Dilution	Yes	No	$10^{4.24}$	[23]
2500 p.p.m. (N	NaDCC)†	2 min	10% plasma	Infectivity	$Na_2S_2O_3$	Yes	Yes	2×10^5	[8]
5000 p.p.m. (N		1 min	50% plasma	Infectivity	Dilution	No	No	7·0 log $TCID_{5}$	[27]
5000 p.p.m. (N	NaDCC)†	2 min	50% blood	Infectivity	$Na_2S_2O_3$	Yes	Yes	2×10^5	[8]
5000 p.p.m. (N	granules)†	2 min	50% blood	Infectivity	$Na_2S_2O_3$	Yes	Yes	1×10^5	[8]

* The concent ı of available chlorine quoted is the stock concentration, not the concentration in the disinfectant test mix except where indicated by †.

† Concentratic available chlorine quoted in the reference is that in the disinfectant test mixture. NaOCl: sodium hypochlorit DCC: sodium dichloroisocyanu $Na_2S_2O_3$: sodium thiosulphate. RT: reverse transcriptase.

of the experiments. Prior determination of the available chlorine content in the product tested or the resulting solutions is a necessary requirement for all tests with hypochlorite solutions since the available chlorine content decreases with time, especially if the solutions have been kept in the warm and exposed to light [12, 14, 15]. Bloomfield and colleagues [16] are the only other group who have both examined the inactivation of HIV by sodium hypochlorite and reported titration of their test solutions to determine the precise concentration of available chlorine.

The neutralization of disinfectant activity without cytotoxicity after the exposure period may be approached in several ways, such as by dilution or by the addition of a chemical neutralizer. Sodium thiosulphate has been used widely as a neutralizing agent of sodium hypochlorite in bactericidal studies [17–22]. Bloomfield and colleagues [16] used sodium thiosulphate plus dilution in saline (500 fold) for 10 min to achieve neutralization of NaDCC granules prior to sampling small aliquots for HIV activity. We also used sodium thiosulphate to neutralize the disinfectant in our test mixtures. Nevertheless, some disinfectant/neutralizer concentrations which did not have a toxic effect on the cells were toxic to the virus at high levels of available chlorine, indicated by a marked difference between the titre of the untreated control and that of the adsorption control. Although we have a good means of measuring cytotoxicity, we do not have a standard method of preventing it.

Virucidal disinfectant tests must, as far as possible, be applicable to the *in vivo* situation. If feasible, the titre of virus tested should equal or preferably exceed the maximum titre found in blood and body fluids. The maximum amount of infectious HIV detected in blood is approximately 10^4 virions per ml [23, 24]. The interpretation of disinfectant efficacy becomes difficult if the test contained a low quantity of virus. In the Bloomfield study on NaDCC granules [16], the sample examined for viral infectivity would have contained a maximum of only 100 viral particles. This low bioburden was cited by the authors as an explanation for their findings of complete HIV inactivation which differed from their previous experiments in a bacteriological test system. These suggested that 10000 p.p.m. available chlorine may be insufficient for decontamination of bacterial pathogens unless applied at v/v ratios of at least nine parts hypochlorite solution to one part blood [25].

When chlorine reacts with proteinaceous material, some of the chlorine is taken up to form *N*-chloro compounds, giving a reserve of combined chlorine, and some is lost through oxidative processes. We have shown that the level of available chlorine is reduced rapidly in the presence of additional protein, with a dramatic decrease in the amount of the active agent available to inactivate the virus. This would explain our virucidal results and those of Bloomfield and colleagues [16] which indicate that a high quantity of available chlorine is required for inactivation of HIV in high levels of blood. In some clinical situations, a low ratio of disinfectant to blood may be used. We have shown that, in the presence of 80% blood, 1000 p.p.m. available chlorine is not effective at inactivating HIV, even after 1 h. Clotted blood poses the additional problem of penetration by the disinfectant; we did not attempt to examine such a model in this study.

The organic load used in disinfectant testing is an important consideration when

interpreting results. The protein challenge in studies from other workers varies between no protein and 50% human plasma (Table 5) and we have shown dramatic differences in the rate of inactivation in the presence of low and high serum levels and blood. Our observations of rapid HIV inactivation by sodium hypochlorite in low protein conditions (8% serum) agree with studies by other workers [16, 26–30]. Hence, in a clean environment, chlorine releasing agents should be rapid and effective disinfectants against HIV.

Our results demonstrate clearly the limitations of chlorine based disinfectants against HIV in conditions simulating soiled/dirty contamination. A concentration of 5000 p.p.m. available chlorine may be insufficient for inactivation of HIV in the presence of high levels or organic matter and guidelines recommending this concentration for the disinfection of blood and body fluid spillages should be reconsidered. At a concentration of 10000 p.p.m. available chlorine, the safety margin under conditions of high organic loading may not be excessive. The dramatic difference in the efficacy of sodium hypochlorite in the presence of a high protein load in the form of serum compared to blood is not unexpected, since only 39% of the total protein content of whole human blood is composed of serum. Satisfactory disinfection of HIV (quantified as a three to four log reduction) by sodium dichloroisocyanurate at concentrations of 50 p.p.m. and 2500 p.p.m. available chlorine for clean (0·9% saline) and dirty (0·9% saline containing 10% v/v plasma) conditions respectively was reported by Bloomfield and colleagues [16]. Nevertheless, a final concentration of 5000 p.p.m. available chlorine was required in the presence of 50% blood to produce total inactivation off HIV within 2 min. This emphasizes not just the caution which should be applied in the extrapolation of data form tests in serum to the *in vivo* situation in blood, but also the necessity for thorough cleaning to remove proteinaceous material prior to disinfection.

We have highlighted some of the important factors to be considered when assessing or advising on the use of hypochlorite solutions as disinfectants. Some of the problems encountered when using hypochlorite solutions may be reduced with the use of NaDCC granules. These dissolve in water to give hypochlorous acid, and are stable for much longer than hypochlorite solutions. They produce higher levels of available chlorine and contain effectively any spillages [14, 31]. Another alternative is the use of powders consisting of a mixture of a chlorine-releasing agent with a highly absorbent acrylic resin which absorbs liquid to form a semi-solid gel. although Coates and Wilson [32] found them to be better suited to watery spillages than blood. These products should be avoided when disinfecting liquids of low pH (urine and other acidic fluids) in poorly ventilated conditions, due to the release of chlorine gas.

Standard virucidal tests exist in some countries (the AFNOR test in France, the DGM tests in Germany and the dried carrier tests required for EPA approval in the US), but there are no agreed UK protocols in the medical field and no internationally agreed standards. Discussions are underway at the Comité Européen de Normalisation (CEN) to provide standard European test methods for the virucidal efficacy of chemical disinfectants and antiseptics in the medical and veterinary field. It is hoped that many of the problems in interpretation of the results by different workers would be addressed by the use of such standardized test methods.

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