



# Evaluation of the antimicrobial activity of a new super-oxidized water, Sterilox<sup>®</sup>, for the disinfection of endoscopes

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**Summary:** The antimicrobial activity of a new super-oxidized water, Sterilox<sup>®</sup>, has been tested against *Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulare*, *Mycobacterium chelonae*, *Escherichia coli* (including type 0157), *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Bacillus subtilis* var *niger* spores, methicillin-resistant *Staphylococcus aureus*, *Candida albicans*, poliovirus type 2 and human immunodeficiency virus HIV-1. Under clean conditions, freshly generated Sterilox was found to be highly active against all these micro-organisms giving a 5 log<sub>10</sub> (99.999%) or greater reduction in two minutes or less.

**Keywords:** Sterilox; endoscopes; high level disinfection.

## Introduction

Thorough cleaning followed by sterilization, or at least high level disinfection, is an essential prerequisite for the re-use of heat sensitive medical and surgical instruments such as flexible fiberoptic endoscopes.<sup>1,2</sup> Gaseous sterilization with ethylene oxide is an option but few hospitals have such facilities and the process, with subsequent aeration, takes more than 24 h. It is therefore impractical as a between-patient procedure. Disinfectants such as 2% glutaraldehyde are consequently widely used for this purpose but aldehydes are toxic, irritant and sensitizing to the skin, eyes and respiratory

tract.<sup>3,4</sup> Furthermore, glutaraldehyde is only slowly effective against mycobacteria and spores and the disinfectant manufacturers advise contact times varying from 20 min for high level disinfection to 10 h for sterilization. A minimum contact time of 20 min has been recommended by the British Thoracic Society<sup>5</sup> for bronchoscopes but even longer periods of up to 120 min have been proposed for *Mycobacterium avium-intracellulare*.<sup>6,7</sup> The costs of additional endoscopes, required due to long disinfection contact times, are substantial.

Of even greater importance are the health hazards associated with handling these aldehydes and the substantial compensation payments for allergies, such as occupational asthma and dermatitis, that have been incurred.<sup>3,4</sup> The newer quarternary ammonium disinfectants, such as Sactimed-1-Sinald<sup>®</sup>, are comparable to glutaraldehyde in their bactericidal

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but they are ineffective against some enteroviruses and spores. Other disinfectants, such as 0.2% peracetic acid (Steris®), 0.35% peracetic acid (Nu-Cidex®) and chlorine dioxide (Tristel®; Dexit®; Medicide®) have been shown to be effective against a wide range of microorganisms including mycobacteria and spores after only 5–10 min exposure.<sup>10–12</sup> However, these are more corrosive and damaging to endoscopes and processing equipment than glutaraldehyde,<sup>13</sup> are still associated with health risks and are more expensive than glutaraldehyde.

Although the development of automated washer-disinfectors with vapour containment or extraction systems has substantially reduced exposure risk,<sup>14</sup> it would still seem appropriate to select a disinfectant which is less irritant and yet is still effective and non-damaging to instruments and processing equipment, if this costs no more than presently used disinfectants.<sup>13</sup>

We report here our experiences with a new disinfectant, Sterilox®, a water containing a mixture of oxidizing species ('super-oxidized water'). The main product is HOCl (hypochlorous acid) at a concentration of about 144 mg/L and Cl<sub>2</sub> (chlorine). This disinfectant is generated at the point of use by passing a saline solution over coated titanium electrodes at 9 amps. The product generated has a pH of 5.0–6.5 and an oxidation reduction potential (redox) of >950 mV. The resultant solution, Sterilox, is claimed by the manufacturer to be non-corrosive and non-damaging to endoscopes and processing equipment.

## Materials and methods

### Sterilox disinfectant

This is the product of the electrolysis of an aqueous saline solution passed over a mixture of proprietary catalysts to give a mixture of oxidizing species, particularly OCl<sup>-</sup>. The disinfectant was produced as required on each test day; the apparatus (supplied by Sterilox Medical Ltd, Abingdon, UK) was operated to give a

final solution redox potential of >950 mV as recommended by the company. Prior to any testing procedure, the possible interfering effects of the disinfectant was established, e.g. effects of neutralizing solution, diluents etc. In none of the investigations reported here was there evidence of interference with the test procedures.

### Efficacy tests: mycobactericidal activity

The method used was that of Griffiths *et al.*<sup>15,16</sup> Suspensions of *M. tuberculosis* H37Rv (NCTC 7416), *M. chelonae* (a glutaraldehyde-resistant strain isolated from an endoscope washer disinfectant, HIRL<sup>15</sup>) and *M. avium-intracellulare* (clinical isolate), all cultured on Middlebrook 7H11 agar (Becton Dickinson, Cowley, Oxford, UK), were prepared by harvesting and mixing each strain with 10 mL sterile distilled water and glass beads. The mixture was shaken for 5 min, then allowed to settle for 30 min, the supernatant was removed and allowed to settle for a further 2 h. The resulting supernatant was used in the disinfectant tests. To simulate organic loading, each bacterial suspension was prepared in the presence of 10% v/v defibrinated horse serum (total protein 7.0 g/dL). Freshly generated Sterilox with a redox of >950 mV (900 µL) was added to 100 µL of the test suspension (with or without organic loading) and biocidal activity allowed to proceed for periods up to 1 h. At each sampling time (1, 4, 10, 20 and 60 min) 10 µL of the mixture was removed and added to 990 µL of a neutralization/recovery system. For Sterilox and sodium dichloroisocyanurate (NaDCC) the neutralization recovery system was nutrient broth (Oxoid No. 2, Oxoid, Basingstoke, UK) containing 1% w/v sodium thio-sulphate and 0.75% w/v lecithin/Tween 80 mixture (5 g lecithin in 50 g Tween 80). This solution has been shown to be effective in inhibiting biocidal activity of Sterilox and NaDCC without inhibiting the growth of surviving test organisms. For glutaraldehyde, a double strength nutrient broth (Oxoid No. 2) containing horse plasma was used as the neutralizing recovery medium. Dilutions were

prepared to  $10^{-3}$  in 900  $\mu\text{L}$  Ringer's solution with duplicate 100  $\mu\text{L}$  aliquots of each dilution being plated onto Middlebrook 7H11 agar plates. These were incubated at 30°C or 37°C for up to six weeks (depending on the choice of test strain) and colony forming units counted. All tests were carried out in duplicate. The results with Sterilox solution were variously compared with the effect of 2% glutaraldehyde and sodium dichloroisocyanurate (a chlorine-releasing agent) included in the present testing protocol as it is often used to destroy glutaraldehyde-resistant strains of *M. chelonae* in endoscope washer-disinfectors.<sup>16</sup>

#### **Efficacy tests: virucidal activity**

Poliovirus type 2 Sabin vaccine strain was cultured in Vero cells maintained in 199 medium (Sigma, Poole, UK) with 1% v/v foetal calf serum (FCS, total protein 6.4 g/dL). The supernatant ( $10^{5.5}$  TCID<sub>50</sub>/mL) was used for virucidal tests. Human immunodeficiency virus (HIV-1; cell adapted strain 2036 Cambridge) was grown in MT-2 cells maintained on RPM 1640 medium (Sigma) containing 10% v/v FCS. The resulting cell culture fluid ( $10^{5.5}$  TCID<sub>50</sub>/mL) was used in the disinfection tests. Inactivation studies were thus carried out using viral suspensions in the presence of organic material (approximately 1% v/v FCS for poliovirus and 10% v/v FCS for HIV). Aliquots of each virus suspension were mixed with freshly generated Sterilox (redox >950 mV and used within 5 h of preparation) in the ratios of 1:1, 1:5 and 1:10 virus suspension: disinfectant. The viruses were exposed to the disinfectant for periods up to 20 min with samples being taken at appropriate times into foetal calf serum (final 2% or 10%; shown to inhibit any residual Sterilox without interfering with virus growth – data not shown) before being assayed by the 50% endpoint (TCID<sub>50</sub>) method. Test cultures were observed for the development of cytopathic effect (cpe) with results after three days (poliovirus) and seven days (HIV) being used for calculations. Tests were carried out in duplicate.

#### **Efficacy tests: sporicidal, bactericidal and fungicidal**

Other micro-organisms tested against Sterilox were:

- (1) Three strains of *Escherichia coli* (type strain NCTC 9001; type 0157 NCTC 12900; type 0157 clinical isolate from a case of haemolytic uraemic syndrome<sup>17</sup>) were grown overnight on Diagnostic Sensitivity Test (DST; Oxoid) agar plates. Culture plates were flooded with 5 mL phosphate buffered saline (PBS) and the growth gently scraped off into suspension. The recovered suspension was adjusted to 20 mL with PBS, gently agitated for two minutes and allowed to stand for five minutes before removing the supernatant. Each organism was washed three times with PBS by centrifugation (to remove contaminating organic material derived from the culture medium) and the final suspension in PBS used for inactivation studies. Assays of pre- and post-inactivation samples were conducted by plating on to DST agar plates.
- (2) A recent clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) phage-type 15 was prepared for use in the disinfection tests and assayed as described above for *E. coli*.
- (3) *Pseudomonas aeruginosa* NCTC 6749 was grown as an overnight nutrient broth suspension and used without washing of the cells. Assays were conducted by plating on to tryptone soya agar (Oxoid) plates.
- (4) *Enterococcus faecalis* NCTC 775 was grown overnight as a nutrient broth suspension and used without washing of the cells. Assays were conducted by plating on to tryptone soya agar plates.
- (5) *Bacillus subtilis* var *niger* NCTC 10073 suspension containing  $>10^7$  spores/mL (prepared by heat shocking the bacterial suspension at 80°C for one minute immediately before use) was used without washing the cells. Assays were conducted by plating onto tryptone soya agar plates.
- (6) *Candida albicans* (a recent clinical isolate)

was grown for two days at 37°C on Sabouraud's agar (Oxoid) and the growth harvested and washed as described for *E. coli* above. Assays were conducted by plating onto Sabouraud's agar plates.

Disinfection testing was carried out by adding 1 mL of each micro-organism suspension to 9 mL of freshly prepared Sterilox (redox >950 mV) and mixing thoroughly (final 1:10 organism suspension disinfectant). For investigations of the microbicidal effect with an organic load, the reaction mixture also contained serum (horse or calf, total protein 7.0 g/dL) at varying levels, i.e., 1, 5 and 10%. Samples of reaction mixtures (1 mL) were taken at intervals during the test period (up to two hours) into 9 mL of neutralizer (as for mycobacteria) to prevent further inactivation taking place, plated and incubated as appropriate. Inactivation tests with the above organisms were carried out in triplicate.

#### *Surface test with B. subtilis var niger*

A preliminary evaluation of the efficacy of Sterilox (compared with 2% glutaraldehyde) in reducing the numbers of spores of *B. subtilis var niger* in a surface test was carried out as follows. Spores, deposited onto aluminium foil (prepared according to DoH specification TSS/S/330.012 and obtained from Steriseal Ltd, Redditch, UK) were immersed in 10 mL of either freshly prepared Sterilox or 2% glutaraldehyde. At specific time intervals, i.e., 1, 5, 10, 20, 30, 60 and 120 min, the spore strips were aseptically removed and placed in 10 mL of the appropriate recovery neutralizer broth (formulations as above). Five spore strips were immersed in separate universal containers for each time interval. One of the five strips was agitated with glass beads in the recovery broth and enumeration carried out as previously described above. The remainder of the dilutions prepared together with the other four strips were incubated at 37°C and examined for growth up to 14 days. Spores were recovered in the same manner from three untreated strips in order to determine the pre-disinfection challenge.

## Results

### *Mycobactericidal activity*

A  $>5 \log_{10}$  reduction in *M. tuberculosis* is indicative of the suitability of a disinfectant for high-level disinfection. It will be seen from Table I that Sterilox in the absence of organic soiling gave a substantial ( $>5 \log_{10}$ ) reduction of *M. tuberculosis* within one minute of exposure. In comparison, 2% glutaraldehyde gave a similar reduction but only after 20 min exposure. Under conditions of light organic loading (i.e., a final concentration of 1% defibrinated horse serum; 0.5 g/litre protein, in the reaction mixture), Sterilox again out-performed glutaraldehyde by achieving  $>5 \log_{10}$  reduction within one minute exposure compared to 10 min with glutaraldehyde.

When the effect of Sterilox was compared with the action of 2% glutaraldehyde and two concentrations of the chlorine-releasing agent sodium dichloroisocyanurate (NaDCC; 1000 and 10 000 mg/L), a  $>6 \log_{10}$  inactivation of *M. chelonae* (a problematic washer-disinfector isolate resistant to 2% glutaraldehyde) was achieved within one minute under both clean and dirty conditions with Sterilox. In contrast, the poor mycobactericidal performance of 2% glutaraldehyde previously noted (Table I) was confirmed whilst NaDCC (at 1000 mg/L) performed better than glutaraldehyde but not as well as Sterilox (Table II). Inactivation was comparable to Sterilox findings only when NaDCC was used at 10 000 mg/L but intermediary concentrations were not investigated. Similar results were obtained when the three disinfectants were compared using *M. avium-intracellulare* as the test organism (Table III).

### *Virucidal activity*

As shown in Table IV, Sterilox failed to achieve adequate virucidal activity against poliovirus type 2, probably due to the presence of organic loading (as FCS) in the viral preparation, when mixed with Sterilox in equal proportions (1:1). However, with increased volume of the disinfectant (more closely reflecting use conditions

**Table I** Inactivation of *Mycobacterium tuberculosis* by Sterilox and 2% glutaraldehyde

Test conditions	Mean log <sub>10</sub> pre-disinfection count	Mean† log <sub>10</sub> reduction after exposure for			
		1 min	4 min	10 min	20 min
No organic loading					
Sterilox	8.1	>5.1*	>5.1	>5.1	>5.1
2% glutaraldehyde	8.0	1.3	2.8	4.6	>5.0*
1% horse serum					
Sterilox	8.3	>5.3*	>5.3	>5.3	>5.3
2% glutaraldehyde	8.0	0.5	2.2	>5.0*	>5.0

\* Detection limit of test

† Tests carried out in duplicate

**Table II** Comparison of effect of Sterilox, 2% glutaraldehyde and NaDCC on an atypical glutaraldehyde-resistant *Mycobacterium chelonae*

Test conditions	Mean log <sub>10</sub> pre-disinfection count	Mean† log <sub>10</sub> reduction after exposure for				
		1 min	4 min	10 min	20 min	60 min
No organic loading						
Sterilox	9.4	>6.4*	>6.4	>6.4	>6.4	>6.4
2% glutaraldehyde	9.1	0	0.1	0.1	0.3	0.3
1000 mg/L NaDCC	8.8	1.8	>5.8*	>5.8	>5.8	>5.8
10 000 mg/L NaDCC	9.2	>6.2*	>6.2	>6.2	>6.2	>6.2
1% horse serum						
Sterilox	9.5	>6.4*	>6.4	>6.4	>6.4	>6.4
2% glutaraldehyde	9.2	0	0	<0.1	<0.1	<0.1
1000 mg/L NaDCC	9.5	<0.1	0.8	3.2	4.8	5.9
10 000 mg/L NaDCC	8.2	>5.2*	>5.2	>5.2	>5.2	>5.2

\* Detection limits of test

† Tests carried out in duplicate

during endoscope disinfection), the effects of the interfering organic material were overcome (quenching of the disinfectant demand exerted by the serum) and inactivation occurred rapidly with a reduction of at least  $4.5 \log_{10}$  within two minutes exposure. A low initial titre of virus and dilution during recovery prevented measurement of reductions in excess of  $4.5 \log_{10}$ .

Inactivation of the HIV strain was rapid at all levels of Sterilox with the presence of serum apparently having little effect. This probably reflects the differing characteristics of the two test viruses.

### Other tests of microbicidal activity

The efficacy of Sterilox against a range of other micro-organisms is shown in Tables V and VI. Table V clearly illustrates that, in the absence of interfering organic material, inactivation was both rapid and complete with at least a  $6 \log_{10}$  reduction for *E. coli* and MRSA and a  $5 \log_{10}$  reduction for *C. albicans* within four minutes exposure. However, it was noticeable that the two laboratory adapted strains of *E. coli* obtained from the National Collection for Type Cultures (i.e., NCTC 9001, the *E. coli* type strain and

Table III Comparison of effect of Sterilox, 2% glutaraldehyde and NaDCC on *Mycobacterium avium*-intracellulare

Test conditions	Mean log <sub>10</sub> pre-disinfection count	Mean† log <sub>10</sub> reduction after exposure for				
		1 min	4 min	10 min	20 min	60 min
No organic loading						
Sterilox	9.5	5.2	>6.5*	>6.5	>6.5	>6.5
2% glutaraldehyde	9.9	0.4	1.2	2.0	3.7	>6.9*
1000 mg/L NaDCC	9.9	<0.1	1.5	3.1	3.7	5.2
10 000 mg/L NaDCC	9.6	>6.6*	>6.6	>6.6	>6.6	>6.6
1% horse serum						
Sterilox	9.2	5.5	>6.2*	>6.2	>6.2	>6.2
2% glutaraldehyde	9.6	1.2	3.8	>6.6*	>6.6	>6.6
1000 mg/L NaDCC	9.5	0.1	0.3	0.5	1.5	>6.5*
10 000 mg/L NaDCC	9.6	3.6	4.8	>6.6*	>6.6	>6.6

\* Detection limits of test

† Tests carried out in duplicate

Table IV Virucidal activity of Sterilox against Poliovirus-2 and HIV-1

Virus	Ratio virus: Sterilox	Mean log <sub>10</sub> pre-disinfection count	Mean† log <sub>10</sub> reduction after exposure for			
			2 min	5 min	10 min	20 min
Polio-2	1:1	5.5	2.0	3.0	2.0	3.0
	1:5	5.5	>4.5*	>4.5	>4.5	>4.5
	1:10	5.5	>4.5*	>4.5	>4.5	>4.5
HIV-1	1:1	5.5	>4.5*	>4.5	>4.5	>4.5
	1:5	5.5	>4.5*	>4.5	>4.5	>4.5
	1:10	5.5	>4.5*	>4.5	>4.5	>4.5

\* Detection limit of test

† Tests carried out in duplicate

NCTC 12900 a genetically modified type 0157 lacking the ability to produce toxin) were more susceptible to Sterilox than the recent clinical isolate of type 0157 which had only received minimal laboratory subculturing. Even so, the latter was totally inactivated within five minutes exposure under clean conditions. As expected, the organic loading (5% calf serum) reduced the rate of killing although this was complete for all *E. coli* strains within 20 min. Again, the two laboratory adapted strains were more susceptible to disinfection by Sterilox than the recent clinical isolate. Interestingly, the organic

loading did not seem to affect the inactivation rate of *C. albicans*.

Table VI shows the results of a comparison of the disinfection efficacy of Sterilox with 2% glutaraldehyde using three other organisms. Both disinfectants were effective in inactivating suspensions of *E. faecalis* and *P. aeruginosa* in the absence of organic loading with complete inactivation within 30 s exposure. However, the biocidal activity of Sterilox against *P. aeruginosa* and *E. faecalis* was slower than 2% glutaraldehyde in the presence of high organic load (10% horse serum). The sporicidal activity of

**Table V** Effect of Sterilox against other micro-organisms at a ratio of 10:1 disinfectant:organism

Test organism	Calf serum	Mean log <sub>10</sub> pre-disinfection count	Mean † log <sub>10</sub> reduction after exposure for								
			0.5 min	1 min	2 min	3 min	4 min	5 min	10 min	20 min	
<i>E. coli</i> NCTC 9001	Absent	8.7	>6.7*	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7
	5%	8.7	-	-	-	-	-	>6.7*	>6.7	>6.7	
<i>E. coli</i> NCTC 12900	Absent	9.0	>7.0*	>7.0	>7.0	>7.0	>7.0	>7.0	>7.0	>7.0	
	5%	9.0	-	-	-	-	-	>7.0*	>7.0	>7.0	
<i>E. coli</i> O157 clinical isolate	Absent	8.8	4.0	4.0	4.0	4.0	>6.8*	>6.8	>6.8	>6.8	
	5%	8.8	<1.8	<1.8	<1.8	1.9	1.9	2.4	4.0	>6.8*	
MRSA clinical isolate	Absent	8.7	>6.7*	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	
	5%	8.7	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.6	>6.7*	
<i>C. albicans</i> isolate	Absent	7.2	>5.2*	>5.2	>5.2	>5.2	>5.2	>5.2	>5.2	>5.2	
	5%	7.2	1.1	1.8	3.7	>5.2*	>5.2	>5.2	>5.2	>5.2	

\* Detection limits of test

† Tests carried out in triplicate

MRSA methicillin resistant *S. aureus***Table VI** Comparison of the effect of Sterilox (10:1 ratio) and 2% glutaraldehyde

Test organism	Disinfectant	Horse serum	Mean log <sub>10</sub> count pre-disinfection	Mean † log <sub>10</sub> reduction after exposure for								
				0.5 min	1 min	2 min	5 min	10 min	20 min	30 min	60 min	120 min
<i>B. subtilis</i> spores	Sterilox	Absent	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
		1%	7.2	2.0	5.2	5.2	7.2	7.2	7.2	7.2	7.2	7.2
		10%	7.5	0.2	0.4	0.5	0.6	0.7	1.6	3.2	7.5	7.5
	2% Glut	Absent	7.9	0.4	0.4	0.5	0.5	0.5	0.9	2.1	3.2	7.9
		1%	7.8	0.2	0.3	0.3	0.3	0.5	0.5	1.7	3.2	7.8
		10%	7.8	0.2	0.3	0.3	0.3	0.4	0.8	1.9	3.0	7.8
<i>E. faecalis</i>	Sterilox	Absent	7.7	7.7	7.7	7.7	7.7	7.7	7.7	-	-	-
		1%	7.7	7.7	7.7	7.7	7.7	7.7	7.7	-	-	-
		10%	7.5	1.2	2.1	4.4	7.5	7.5	7.5	-	-	-
	2% Glut	Absent	7.7	7.7	7.7	7.7	7.7	7.7	7.7	-	-	-
		1%	7.7	7.7	7.7	7.7	7.7	7.7	7.7	-	-	-
		10%	7.7	7.7	7.7	7.7	7.7	7.7	7.7	-	-	-
<i>P. aeruginosa</i>	Sterilox	Absent	7.8	7.8	7.8	7.8	7.8	7.8	7.8	-	-	-
		1%	7.8	5.6	7.8	7.8	7.8	7.8	7.8	-	-	-
		10%	7.9	1.7	1.7	3.2	5.8	7.9	7.9	-	-	-
	2% Glut	Absent	8.0	8.0	8.0	8.0	8.0	8.0	8.0	-	-	-
		1%	8.0	8.0	8.0	8.0	8.0	8.0	8.0	-	-	-
		10%	7.9	7.9	7.9	7.9	7.9	7.9	7.9	-	-	-

† Tests carried out in duplicate

Table VII Efficacy of Sterilox after 24 and 48 h storage

Test organism (organic loading)	Age of Sterilox solution	Mean log <sub>10</sub> pre-disinfection	Mean † log <sub>10</sub> remaining after exposure (min)										
			0.5	1	2	5	10	20	30	60	120		
<i>P. aeruginosa</i> – clean	Fresh	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	
	24 h	7.9	2.0	1.2	0.0	0.0	0.0	0.0	0.0	–	–	–	
	48 h	7.8	3.3	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	
	1% serum	Fresh	7.8	2.2	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–
		24 h	7.9	5.9	3.7	0.0	0.0	0.0	0.0	0.0	–	–	–
		48 h	7.8	6.0	2.9	0.0	0.0	0.0	0.0	0.0	–	–	–
10% serum	Fresh	7.9	6.2	6.2	4.7	2.2	0.0	0.0	0.0	–	–	–	
	48 h	7.9	5.3	5.3	5.0	3.4	0.0	0.0	0.0	–	–	–	
<i>E. faecalis</i> – clean	Fresh	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	
	24 h	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	
	48 h	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	
	1% serum	Fresh	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–
		24 h	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–
		48 h	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–
10% serum	Fresh	7.5	6.3	5.8	3.1	0.0	0.0	0.0	0.0	–	–	–	
	48 h	7.6	5.1	4.6	4.6	1.4	1.8	0.0	0.0	–	–	–	
<i>B. subtilis</i> spores – clean	Fresh	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	
	48 h	7.6	4.5	2.3	1.6	0.0	0.0	0.0	0.0	–	–	–	
	1% serum	Fresh	7.2	5.2	2.0	2.0	0.0	0.0	0.0	0.0	–	–	–
		48 h	7.7	5.6	3.1	2.0	1.3	0.0	0.0	0.0	–	–	–
		48 h	7.7	5.6	3.1	2.0	1.3	0.0	0.0	0.0	–	–	–
	10% serum	Fresh	7.5	7.3	7.1	7.0	6.9	6.7	5.9	4.3	0.0	0.0	0.0
48 h		7.5	7.1	7.0	7.0	6.7	6.7	6.0	4.7	1.9	0.0	0.0	

† Tests carried out in duplicate

Sterilox was far superior to that of 2% glutaraldehyde in the absence of an organic load and in the presence of 1% horse serum. In conditions of high organic soiling (10% serum), Sterilox was slightly more sporicidal than 2% glutaraldehyde but both disinfectants required a contact time of at least an hour to result in a 5 log<sub>10</sub> reduction of bacterial numbers.

### Efficacy tests

#### Effect of ageing of Sterilox

Whilst Sterilox is intended to be generated fresh at point of use, it is possible that there may be residual older disinfectant within the production equipment. Accordingly, it is appropriate that the efficacy of Sterilox solution be measured in the event that there is a delay before fresh disinfectant is available. Evaluations were carried out using *P. aeruginosa*, *E. faecalis* and *B. subtilis* spores using disinfectant stored for 24 and 48 h. Table VII shows that, whilst there

was some slight loss of activity, when tested under clean conditions the disinfectant was still effective within 5 min even when 48 h old.

#### Surface test using *B. subtilis var niger* spores

The results shown in Table VIII indicate that Sterilox is an extremely effective sporicide when used in a surface test under clean conditions. In comparison, the same level of reduction (>5 log<sub>10</sub>) was only achieved with 2% glutaraldehyde after two hours exposure.

### Discussion

There has been a recent interest in the use of super-oxidized water as a disinfectant because of its rapid and highly biocidal activity against a wide range of bacteria.<sup>18</sup> However, the electrolysis of a saline solution using the Super Oxseed alpha 1000 unit (Janix Inc, Kanagawa, Japan) produces a super-oxidized water with a

**Table VIII** Efficacy of Sterilox against *B. subtilis var niger* spores in a surface test

Disinfectant	Pre-count log <sub>10</sub>	Log <sub>10</sub> remaining after (number of tubes showing growth)					
		5 min	10 min	20 min	30 min	60 min	120 min
Sterilox	5.6	0	0	0	0	0	0
2% Glutaraldehyde	5.6	5.6 (5/5)	4.4 (5/5)	4.3 (5/5)	3.9 (5/5)	1.3 (5/5)	0 (0/5)

highly acidic pH of 2.3–2.7 which limits its suitability for many applications, in particular the disinfection of endoscopes. We have studied a different system (Sterilox 2500; Sterilox Medical Ltd) which passes a sodium chloride solution over coated titanium electrodes at 9 amps to produce a super-oxidized water, 'Sterilox' with a redox potential of >950 mV and a pH in the range 5–6.5. This product has been (a) tested for occupational exposure levels of chlorine which was found to be below analytical detection limits (Dr J. Dennis, Bradford University Research Ltd, research report to Sterilox Medical Ltd, 1997) and (b) shown to be non-toxic orally and non-irritant to skin and mucous membranes (Report from Huntingdon Research Life Sciences Ltd to Sterilox Medical Ltd, 1997) using internationally recognized testing protocols satisfying the requirements of the EEC Directive 92/69/EEC (1993). Another area apparently being evaluated is the effect of the disinfectant on the materials used in the construction of the endoscopes, but at this time the authors have not seen the outcome of such investigations.

As shown by Tanaka *et al.*,<sup>18</sup> the addition of bovine serum albumin, at a concentration of 0.5%, reduces the biocidal activity of super-oxidized water. We have confirmed this and agree that in the presence of high organic loading the biocidal activity of Sterilox, as with other chlorine releasing and oxidizing agents, is impaired and thus is not suitable for the disinfection of spillage or grossly soiled instruments. However, when organic loading is substantially reduced by thorough manual cleaning of endoscopes followed by automated washing as is the norm, there was no inhibitory effect on the biocidal activity of Sterilox against mycobacteria and *B. subtilis* spores, the most resistant organisms we tested.

All the standard methods for testing disinfectants are based on the need to demonstrate activity in the presence of gross organic soiling. However, the presence of organic contamination is of little relevance to the proposed use of Sterilox as a single use disinfectant wash after endoscopes have been through a thorough validated cleaning process. Any organic material present in or on the endoscope after cleaning will be reduced to a concentration of less than 0.1% protein by the large volume (usually 10–20 litres) of the Sterilox used in the disinfection process. Furthermore, the residual organic material will be rapidly saturated and its inhibitory effect quenched by the disinfectant flow. We propose, therefore, that the tests used in this study are appropriate for this specific method of usage of a disinfectant.

Sterilox has shown promising potential for use in automated washer-disinfectors where the quality of the initial wash and the single use of the disinfectant can be guaranteed. As a consequence of this approach, i.e., to use 10–20 litres of fresh Sterilox for each disinfection cycle, we have chosen as an appropriate *in vitro* bactericidal test a concentration of one part of micro-organism suspension to be exposed to ten parts of Sterilox. We believe that using the disinfectant in this way, this 1:10 ratio is a very conservative estimate of the actual situation achieved during use. Using this 1:10 ratio, we have shown that, under clean conditions, Sterilox rapidly inactivates mycobacteria (*M. tuberculosis*, *M. avium-intracellulare* and *M. chelonae*), MRSA, *E. faecalis*, *P. aeruginosa*, *B. subtilis var niger* spores, *E. coli* (including 0157) and *C. albicans*. In addition, Sterilox has been shown to be effective against *B. subtilis* spores on aluminium strips (surface test).

The introduction of endoscope washer-disinfectors has greatly improved processing standards and has reduced the likelihood of staff exposure to irritant chemicals.<sup>14</sup> However, several new problems have arisen with automated processing which are difficult to overcome. If the washer-disinfectant is not disinfected, at least on a sessional basis, or the rinse water used to remove toxic disinfectant residues is not sterile, infection or pseudo-infection may ensue.<sup>19</sup> This is a major problem in bronchoscopy, where the misdiagnosis of tuberculosis has occurred because the bronchoscope has become contaminated with acid-fast *M. chelonae* from the rinse water<sup>20</sup> some strains of which are highly resistant to glutaraldehyde.<sup>16</sup>

This study shows that a glutaraldehyde-resistant washer-disinfectant isolate of *M. chelonae* was highly susceptible to Sterilox and that this could, therefore, be used for disinfecting washer-disinfectant filters and other rinse water pathways. It is also likely that Sterilox will prove less damaging to the processors than some of the chlorine-releasing and oxidizing agents currently used. Furthermore, as with other disinfectants, the water used to wash out disinfectant residues must be of a suitable microbiological quality. Sterile, or at least bacteria-free, rinse water is required for bronchoscopes and all invasive endoscopes. An advantage of the Sterilox system is that it has been shown that it is possible to produce bacteria-free wash water by automatically mixing the disinfectant with potable water (final 2% Sterilox in wash water) to achieve this (Dr R. Morris, report to Sterilox Medical Ltd, 1996). The effect on biofilms, or the organisms present within, has not been investigated in the present study.

There is now a greater awareness of the irritancy and sensitization problems associated with the use of glutaraldehyde and other aldehyde-based instrument disinfectants. In the UK, the Control of Substances Hazardous to Health (COSHH) regulations require an assessment of health risks associated with processing and, where possible, to substitute a safer alternative provided it is effective. In response to health and safety requirements, a number of alternative disinfectants have emerged which

are claimed to be non- or less irritant and as, or more, effective than glutaraldehyde. These include improved quaternary ammonium compounds (e.g. Sactimed Sinald, Dettol ED®), peroxygen products (Virkon®), peracetic acid (Steris, Nu-Cidex) and chlorine dioxide (Tristel, Dexit and Medicide). Sterilox could be another alternative for glutaraldehyde if field trials confirm it is compatible with instruments and processing equipment.

The manufacturer of the Sterilox system emphasizes that Sterilox can only be generated and stored using the apparatus provided. To ensure full microbicidal activity, all production criteria, i.e., generating current (9 amps), voltage across the cells (9 volts), redox potential (1000 mV) and pH (5.5), must be met before the disinfectant is made available to the user. Since Sterilox is generated on healthcare premises at the point of use, it could be argued that there may be a need for confirmation of the biocidal efficacy of the solution. This is in contrast to other disinfectants which may have their efficacy and stability checked on a batch basis before release. Confirmation of the microbicidal activity of Sterilox at point of production is assessed on installation and may be periodically monitored from then on by biological tests or by determining available chlorine levels ( $\geq 140$  mg/L).

There is another important issue which may affect efficacy, namely the ageing of the solution in pipework and reservoirs, particularly if the endoscope washer-disinfectant is some way from the generator and there is a delay of more than 24 h between processing sessions. It is, therefore, necessary to ensure that the system is purged completely with freshly generated product as part of the daily routine. However, data presented in this report indicate that Sterilox maintains its efficacy for at least 24 h after generation (Table VII).

This study has examined the microbicidal efficacy of Sterilox against a range of bacteria, viruses and fungi which are indicative of the types of organisms that may be expected to be encountered during the use of flexible endoscopes. In particular, several species of

*Mycobacterium* have been investigated, including an atypical endoscope isolate of a glutaraldehyde-resistant strain of *M. chelonae*. The efficacy tests described here have shown that freshly generated Sterilox is highly and rapidly effective in killing spores, mycobacteria and a wide range of other potentially pathogenic micro-organisms associated with diagnostic and therapeutic endoscopy procedures. Of particular importance is the shortened contact time for *M. tuberculosis* and *M. avium-intracellulare* in marked contrast to the currently recommended contact time of 20–60 min for 2% glutaraldehyde. In view of the COSHH regulations, and providing field trials substantiate that (a) the efficacy of Sterilox during routine use is identical or similar to the *in-vitro* tests described here, (b) that the disinfectant is compatible with instruments and processor components, and (c) it is cost effective, Sterilox could be an attractive alternative to glutaraldehyde and should be considered alongside other agents for the disinfection of endoscopes and heat-labile equipment.

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## References

1. Babb JR. Disinfection and sterilisation of endoscopes. *Current Opinion in Infectious Diseases* 1993; 6: 532–537.

2. Medical Devices Agency. Decontamination of endoscopes. *Device Bulletin* 9607 1996. Department of Health.
3. Taylor EW, Mehtar S, Cowan RE *et al.* Endoscopy: disinfectants and health. Report of a meeting held at the Royal College of Surgeons of England, February 1993. *J Hosp Infect* 1994; 28: 5–14.
4. Cowan RE, Manning AP, Ayliffe GAJ *et al.* Special report: aldehyde disinfectants and health in endoscopy units. *Gut* 1993; 34: 1641–1645.
5. Woodcock A, Campbell I, Collins JV *et al.* Bronchoscopy and infection control. *Lancet* 1989; ii: 270–271.
6. Uttley AH, Simpson RA. Audit of bronchoscope disinfection: a survey of procedures in England and Wales and incidents of mycobacterial contamination. *J Hosp Infect* 1994; 26: 301–308.
7. Rutala WA. APIC guideline for selection and use of disinfectants. *Am J Infect Control* 1990; 18: 99–117.
8. Holton J, Nye P, McDonald V. Efficacy of selected disinfectants against mycobacteria and cryptosporidia. *J Hosp Infect* 1994; 27: 105–115.
9. Nicholson G, Hudson RA, Chadwick MV *et al.* The efficiency of the disinfection of bronchoscopes contaminated *in vitro* with *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare* in sputum: a comparison of Sactimed-1-Sinald and glutaraldehyde. *J Hosp Infect* 1995; 29: 257–264.
10. Holton J, Shetty N, McDonald V. Efficacy of "Nu-Cidex" (0.35% peracetic acid) against mycobacteria and cryptosporidia. *J Hosp Infect* 1995; 31: 235–237.
11. Lynam PA, Babb JR, Fraise AP. Comparison of the mycobacterial activity of 2% alkaline glutaraldehyde and "Nu-Cidex" (0.35% peracetic acid). *J Hosp Infect* 1995; 30: 237–240.
12. Bradley CR, Babb JR, Ayliffe GA. Evaluation of Steris system 1 peracetic acid endoscope processor. *J Hosp Infect* 1995; 29: 143–151.
13. Babb J, Bradley CR. A review of glutaraldehyde alternatives. *Br J Theatre Nursing* 1995; 5: 20–21.
14. Bradley CR, Babb JR. Endoscope decontamination: automated vs manual. *J Hosp Infect* 1995; 30 (Suppl): 537–542.
15. Griffiths PA, Babb JR, Fraise AP. *Mycobacterium terrae*; a potential surrogate for *M tuberculosis* in a standard disinfection test. *J Hosp Infect* 1998; 38: 183–192.