An evaluation of the use of chlorine dioxide (Tristel One-Shot) in an automated washer/disinfector (Meditor) fitted with a chlorine dioxide generator for decontamination of flexible endoscopes

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Summary: Microbiological tests were carried out to evaluate a new chlorine dioxide sterilant: Tristel One-Shot. Preliminary in vitro suspension tests showed that solutions containing around 140 ppm chlorine dioxide achieved a reduction factor exceeding 10⁶ of Staphylococcus aureus in 1 min and of Bacillus subtilis spores in 2.5 min in the presence of 3 g/L bovine albumin. Subsequent tests evaluated the effectiveness of Tristel One-Shot in a Medivator washer/disinfector fitted with a Tristel Generator for processing flexible endoscopes. Each test run involved three stages. In the first, the instrument and air–water channels of a gastroscope were inoculated with a suspension of Pseudomonas aeruginosa (10⁸ cfu/ml) in 10% sodium glutamate and serum (0, 5 or 10%) and then drained, partially dried, and saline flushed through for total viable counts (TVCs). In the second stage, the channels were re-inoculated with test organisms; detergent was flushed through the channels which were then brushed; and saline was flushed through for TVCs. In the third stage, the channels were re-inoculated; detergent was flushed through the channels which were then brushed; the endoscope was processed in the Medivator; and saline was flushed through for TVCs. Carrying out all three stages enabled determination of (1) the contribution played by manual cleaning of channels prior to processing in the Medivator, and (2) the combined effect of manual cleaning followed by processing. Two series of test runs were done. In the first, the Tristel Generator was set to generate 230 ppm chlorine dioxide, and in the second 150 ppm. In the first, cleaning followed by processing in the Medivator consistently achieved a >10⁵-fold reduction of test organisms, and in the second a >10⁴-fold reduction. Pre-cleaning of channels was very important—when done the initial concentration of serum in the inoculum (0–10%) had no affect on the results obtained after processing.

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Keywords: Bactericidal tests; chlorine dioxide; Tristel One-Shot sterilant; Tristel Generator; Medivator automated washer/disinfector; flexible endoscopes; video gastroscope.

Introduction

Flexible endoscopes cannot withstand the high temperatures and pressure changes of autoclave cycles and hence are usually decontaminated by cleaning followed by high-level disinfection with a sterilant. Glutaraldehyde was introduced in 1963¹ and rapidly became the first-line agent for the disinfection of endoscopes. Today it is still the most commonly used disinfectant in endoscopy units in the UK. Whilst it is an excellent disinfectant in many respects, it is far from perfect. One of the main problems of glutaraldehyde is that it is toxic,
irritant and allergenic and surveys suggest that the prevalence of symptoms associated with glutaraldehyde exposure is high among staff working in endoscopy units. Until January 1998 glutaraldehyde had an Occupational Exposure Standard (OES) of 0.2 ppm expressed as a 15 min reference period.

However, because no safe exposure limit for glutaraldehyde could be identified, the Health and Safety Executive withdrew the OES and replaced it with a Maximum Exposure Limit (MEL) at 0.05 ppm for both the long-term exposure limit (8 h time weighted average reference period) and the short-term exposure limit (15 min reference period). Exceeding a MEL violates COSHH Regulations and the Health and Safety Executive advises that 'wherever possible, glutaraldehyde should be replaced by a less hazardous substance. For a number of uses, including disinfection of some endoscopes, there are many safer alternatives available'.

Another problem with glutaraldehyde is that its microbicidal activity is too slow for practical purposes against some organisms that cause problems in endoscopy. Glutaraldehyde kills high numbers of Mycobacterium tuberculosis within 20 min and lower numbers within 5–10 min. However, M. avium-intracellulare is much more resistant and killed only after 60–75 min. Bacterial spores are very resistant and the killing time for some is 3 h or longer and the manufacturer of Cidex (a commercial preparation of glutaraldehyde) stipulates 10 h for sterilization. Cryptosporidium parvum oocysts are also very resistant remaining viable and infectious after 10 h contact with glutaraldehyde. Unfortunately, the same flexible endoscope may have to be used many times during a list necessitating a quick reprocessing time between procedures—far shorter than is required to kill these resistant organisms or achieve sterilization. Consequently, thorough pre-cleaning is essential to remove as many organisms as possible as well as organic material which protects them. Cleaning can achieve at least a 10^3–10^4-fold reduction in the microbial contamination load.

A further problem with glutaraldehyde arises with endoscope washer/disinfector isolates of M. chelonae and only slowly effective against M. kansasii. These organisms can be deposited on endoscopes during processing which has led to misdiagnosis of tuberculosis due to acid-fast bacilli being found in bronchial lavage samples taken during bronchoscopy.

In recent years, various alternatives to glutaraldehyde have been developed. These include peracetic acid e.g. Steris, Nu-Cidex, and PeraSafe; chlorine dioxide e.g. Tristel, ‘super-oxidized water’ e.g. Sterilox; and gas plasma e.g. Sterrad. All these alternatives to glutaraldehyde have advantages and disadvantages so it is up to individual endoscopy units to decide which best matches their needs and resources.

In the present study, microbiological tests were carried out to evaluate the effectiveness of a new single-use, low concentration chlorine dioxide formulation (Tristel One-Shot) used in an automated washer/disinfector (Medivator) for processing flexible endoscopes. The aims of the study were to determine the levels of chlorine dioxide that consistently achieved (a) ≥10^5-fold reductions (b) ≥10^6-fold reductions of the test organism inoculated into the endoscope channels under ‘clean’ (0% serum) and ‘dirty’ (5% or 10% serum) conditions.

Materials and methods

Site of study

The study was carried out at The Royal Oldham Hospital with the help and co-operation of the Endoscopy Unit, the Microbiology Laboratory and the Occupational Health & Safety Service.

Test organisms

The test organisms used in in vitro suspension tests were Staphylococcus aureus NCTC 4163 and Bacillus subtilis NCTC 10073 spores which were prepared by the method of Beeby and Whitehouse. The test organism used to inoculate endoscopes was Pseudomonas aeruginosa NCTC 6749. An 18 h culture in Tryptone Soya Broth was used to
prepare a standardized suspension containing $10^8$ colony forming units (cfu)/ml in 10% w/v sodium glutamate (L-glutamic acid sodium salt: BDH) and horse serum (0%, 5% or 10%).

**In vitro suspension tests**

Preliminary *in vitro* suspension tests were carried out by the British/European standard method BS EN 1276: 1997 under simulated clean conditions (organic challenge: 0.3 g/L bovine albumin) and dirty conditions (3 g/L bovine albumin).

**Endoscope used in tests**

The endoscope used in tests was a Pentax EG-2940 Video Gastroscope loaned from the E3 Endoscopy Theatre, Royal Oldham Hospital. This gastroscope has three channels: (1) instrument, (2) air, and (3) water. The air–water channels have a common air–water nozzle at the distal end of the insertion tube, and a common air–water port on the PVE (Pentax Video Endoscope) connector. The instrument channel has three sections: (1) suction nipple on PVE connector → umbilical cable → suction control valve on control body, (2) suction control valve → instrument channel inlet on control body, (3) instrument channel inlet → insertion tube → outlet at distal end of insertion tube. A ‘dog-leg’ exists between the instrument channel inlet and a junction below where the channel to the insertion tube is joined by that from the suction control valve. The air–water channels each have two sections: (1) air-water port on PVE connector → umbilical cord → air-water feeding valve on control body, (2) air-water feeding valve → insertion tube → air-water nozzle at distal end of insertion tube. The lumen of the instrument channel is wider than that of the air–water channels.

**Endoscope washer/disinfector used in tests**

The endoscope washer/disinfector used in tests was a Medivator DSD-91E fitted with a Tristel generator for production of Tristel One Shot sterilant (The Tristel Company Ltd, Cambs., CB8 7NY, UK). The programme cycle used was the standard cycle for flexible endoscopes which has five stages: (1) generation of Tristel by the Tristel generator which takes around 1 min 20 sec, (2) flushing the channels with enzymatic detergent which takes around 1 min 5 sec, (3) disinfection with Tristel involving: (a) filling the load chamber; (b) a time controlled complete immersion phase of exactly 5 min, and (c) emptying the chamber which, altogether, takes around 13 min 5 sec, (4) a first rinse with filtered water which takes around 6 min 45 sec, and (5) a second rinse with filtered water which takes around 6 min 45 sec. Thus the cycle time is around 29 min.

**Test run stages**

Each test run involved three stages. In the first stage, the channels were inoculated, allowed to equilibrate for 10 min, and then drained gravitationally. Sterile saline was flushed up and down the channels several times, collected in sterile bottles and total viable counts (TVCs) done (see below). The purpose of this stage was to determine TVCs in channel washings after inoculation but no cleaning or disinfection. In the second stage, the channels were re-inoculated, equilibrated and drained as before. They were then cleaned by flushing detergent through followed by brushing. Saline was then flushed through and collected for TVCs as before. The purpose of this stage was to determine TVCs in channel washings after inoculation and cleaning but no disinfection. In the third stage, the channels were re-inoculated, equilibrated and drained as before. They were cleaned by flushing detergent through and brushing as before and then processed in the Medivator. Saline was then flushed through and collected for TVCs as before. The purpose of this stage was to determine TVCs in channel washings after inoculation, cleaning and chlorine dioxide disinfection in the Medivator.

**Inoculation of gastroscope channels**

**Instrument channel:** The instrument channel had a volume of approximately 25 ml. Inoculum (40 ml) was prepared by mixing appropriate volumes of culture, 10% sodium glutamate and defibrinated horse serum. This was aspirated into a 60 ml syringe (no needle) and connected to the suction nipple on the PVE connector whilst the distal end of the insertion tube was clamped inside a 50 ml sterile container. The inoculum was expelled into the channel and flushed up and down several times to completely fill the channel. The syringe was then disconnected and the control body held aloft to promote drainage of inoculum from the channel. The gastroscope was then left for 10 min to drain
and partially dry. About 1 ml of inoculum remained in the channel.

**Air–water channels:** The air–water channels had a volume of approximately 15 ml. Inoculum (30 ml) was aspirated into a 60 ml syringe and connected to the air–water port on the PVE connector and the channels inoculated and drained as described above for the instrument channel.

**Cleaning and brushing procedure**

Two detergents were used in test runs. MediGene (MediChem International), described by the manufacturer as a dual enzymatic bactericidal detergent cleaner, was used in 14 test runs. Hospec (Young’s Detergents), a general purpose neutral liquid detergent, was used in one test run. Ten litres of 1% detergent solution were prepared in a baby bath using warm water and the gastroscope immersed. The instrument channel was cleaned by attaching a 60 ml syringe to the suction nipple on the PVE connector and flushing 300 ml (5 × 60 ml) of detergent through. A brush was then passed through the channel (three sections) three times. The air–water channels (two sections) were then cleaned in the same way.

**Processing in the Medivator**

After cleaning, the gastroscope was placed in one of the two Medivator basins and the connectors attached to the gastroscope valves. On pressing the cycle start button, Tristel is generated automatically, pumped through the channels and into the basin until full. At this stage, a sample of Tristel was taken from the basin for assay of chlorine dioxide.

**Sampling procedure**

*Instrument channel:* For each test run, samples were taken after stage 1 (post-inoculation), stage 2 (post-cleaning) and stage 3 (post-processing). The sampling procedure post-inoculation was as follows: a 60 ml syringe (no needle) was connected to the suction nipple on the PVE connector whilst the distal tip of the insertion tube was clamped inside a 50 ml sterile container. The syringe was used to expel the residual inoculum (about 1 ml) from the channel into the container. It was then disconnected, 40 ml of saline aspirated and re-connected. The saline was expelled from the syringe into the channel, flushed up and down several times, and finally expelled into the container. A TVC was then made. The sampling procedure after cleaning and after processing was as above, but omitting the initial steps carried out to expel the residual inoculum.

*Air–water channels:* The sampling procedures were as described for the instrument channel except that the syringe was connected to the air–water port on the PVE connector, and 30 ml of saline was flushed through.

**TVCs on samples**

Six serial 10-fold dilutions of samples (saline flushed through channels) were made in Maximum Recovery Diluent (MRD). One millilitre volumes were inoculated on to dried blood agar plates (in triplicate) and spread using plastic spreaders. Plates were incubated at 37°C for 48 h and colonies counted.

**Assays of chlorine dioxide concentrations in Tristel solutions**

Assays of chlorine dioxide in Tristel solutions were carried out by titration against sodium thiosulphate using the method recommended by (and available from) the supplier.

**Investigation of the possible bactericidal activity of detergents**

Aqueous 1% solutions of MediGene and Hospec were inoculated with 10⁸ cfu/ml of *P. aeruginosa* and viable counts carried out after 0, 5, 10 and 15 min to determine whether there was any decrease in count.

**Investigation of the possible bactericidal activity of sodium glutamate**

An aqueous 10% solution of sodium glutamate was inoculated with 10⁸ cfu/ml of *P. aeruginosa* and viable counts carried out after 0, 1, 2 and 4 h to determine whether there was any decrease in count.

**Tests on a variety of endoscopes that had been used in clinical procedures**

Several types of endoscope were obtained from the endoscopy suite following clinical procedures. The
endoscopes were flushed through with detergent and brushed (as normal) by staff in the endoscopy suite. They were then taken to the research laboratory and processed in the Medivator. Sterile saline was flushed through the channels and 1 ml samples inoculated on to six plates of blood agar. Three were incubated aerobically and three anaerobically, at 37°C for 48 h.

Results

In vitro suspension tests

In vitro suspension test results are shown in Table I. A concentration of 141 ppm chlorine dioxide achieved a reduction of \( S. aureus \) by a factor of \( 10^6-10^7 \) in 1 min under simulated dirty conditions and 146 ppm achieved a reduction of \( B. subtilis \) spores by a factor of \( 10^6 \) in 2.5 min under dirty conditions.

Bacterial challenge in the instrument and air–water channels post-inoculation

Fifteen test runs were carried out (Table II). The first step was to prepare an inoculum consisting of a standardized suspension of approximately \( 10^8 \) cfu/ml of \( P. aeruginosa \) in 10% w/v sodium glutamate and either 0, 5 or 10% horse serum. The TVCs of the inocula prepared were in the range 2.7–7.6 \( \times 10^8 \) cfu/ml. The first stage of each test run was to inoculate the channels, allow them to equilibrate for 10 min and then allow them to drain by gravity. Saline was then flushed up and down the channels and collected for TVCs. Counts after inoculation ranged from 1.7 to 9.8 \( \times 10^7 \) cfu/ml in the instrument channel and from 2.0 to 9.2 \( \times 10^7 \) cfu/ml in the air–water channel. Hence, the bacterial challenge in the instrument channel after inoculation was very similar to that in the air–water channel.

First series of test runs

In the first series of test runs (runs 1–9 in Table II) the Tristel Generator was set to generate a level of around 230 ppm of chlorine dioxide. Nine test runs were carried out with the actual levels of chlorine dioxide generated varying from 201–258 ppm. The second stage of each test run was to re-inoculate the channels with test organisms as before and then to clean them prior to flushing with saline for TVCs. In five of nine test runs, cleaning was carried out by flushing 1% MediGene (detergent) through the channels followed by brushing the channels with a sterile cleaning brush whilst the gastroscope was immersed in a solution of 1% MediGene in water. After cleaning in this manner, the post-flushing and brushing counts ranged from \( 1.6 \times 10^5 \) to \( 2.0 \times 10^5 \) cfu/ml in the instrument channel and from \( 5.8 \times 10^2 \) to \( 3.4 \times 10^5 \) cfu/ml in the air–water channel. Hence, flushing and brushing reduced the inocula in the channels by factors of \( 10^2-10^5 \) with no apparent difference between the instrument and air–water channels. In two of nine test runs cleaning was carried out by brushing alone. In these runs, the counts after brushing ranged from \( 4.2 \times 10^6 \) to \( 6.0 \times 10^6 \) cfu/ml in the instrument channel and from 2.0 \( \times 10^6 \) to \( 7.8 \times 10^7 \) cfu/ml in the air–water channel. Hence, the effect of brushing alone was to reduce the inocula in the channels by between zero and a factor of \( 10^4 \) but with less than a 10-fold

<table>
<thead>
<tr>
<th>Test organism</th>
<th>CI( \text{O}_2 ) (ppm)</th>
<th>Bovine albumin (g/L)</th>
<th>Contact time (min)</th>
<th>Log(_{10}) reduction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S. aureus )</td>
<td>79</td>
<td>0.3</td>
<td>1</td>
<td>3–4</td>
</tr>
<tr>
<td>( S. aureus )</td>
<td>79</td>
<td>3</td>
<td>1</td>
<td>3–4</td>
</tr>
<tr>
<td>( S. aureus )</td>
<td>92</td>
<td>0.3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>( S. aureus )</td>
<td>92</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>( S. aureus )</td>
<td>141</td>
<td>0.3</td>
<td>1</td>
<td>6–7</td>
</tr>
<tr>
<td>( S. aureus )</td>
<td>141</td>
<td>3</td>
<td>1</td>
<td>6–7</td>
</tr>
<tr>
<td>( B. subtilis )</td>
<td>97</td>
<td>0.3</td>
<td>2.5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>( B. subtilis )</td>
<td>97</td>
<td>3</td>
<td>2.5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>( B. subtilis )</td>
<td>120</td>
<td>0.3</td>
<td>2.5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>( B. subtilis )</td>
<td>120</td>
<td>3</td>
<td>2.5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>( B. subtilis )</td>
<td>146</td>
<td>0.3</td>
<td>2.5</td>
<td>6</td>
</tr>
<tr>
<td>( B. subtilis )</td>
<td>146</td>
<td>3</td>
<td>2.5</td>
<td>6</td>
</tr>
</tbody>
</table>
Table II  Effectiveness of cleaning alone, and cleaning followed by disinfection with Tristel One-Shot sterilant in a Medivator endoscope reprocessor fitted with a Tristel generator, in decontaminating the instrument and air–water channels of a gastroscope inoculated with *P. aeruginosa*.

<table>
<thead>
<tr>
<th>Test run</th>
<th>Chlorine dioxide (ppm)</th>
<th>Horse serum (%)</th>
<th>Test Chlorine Horse Bacterial count/ml</th>
<th>Log₁₀ reduction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Instrument after-inoculation</td>
<td>Air–water channel after-inoculation</td>
</tr>
<tr>
<td>1</td>
<td>201</td>
<td>0</td>
<td>3.3 × 10⁸</td>
<td>5.3 × 10⁷</td>
</tr>
<tr>
<td>2</td>
<td>243</td>
<td>0</td>
<td>3.2 × 10⁸</td>
<td>9.2 × 10⁷</td>
</tr>
<tr>
<td>3</td>
<td>258</td>
<td>0</td>
<td>7.2 × 10⁸</td>
<td>8.8 × 10⁷</td>
</tr>
<tr>
<td>4</td>
<td>231</td>
<td>0</td>
<td>3.4 × 10⁸</td>
<td>6.2 × 10⁷</td>
</tr>
<tr>
<td>5</td>
<td>246</td>
<td>5</td>
<td>4.6 × 10⁸</td>
<td>1.7 × 10⁷</td>
</tr>
<tr>
<td>6</td>
<td>238</td>
<td>5</td>
<td>2.7 × 10⁸</td>
<td>5.2 × 10⁷</td>
</tr>
<tr>
<td>7</td>
<td>253</td>
<td>10</td>
<td>5.8 × 10⁸</td>
<td>5.2 × 10⁷</td>
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<tr>
<td>8</td>
<td>250</td>
<td>10</td>
<td>4.2 × 10⁸</td>
<td>6.2 × 10⁷</td>
</tr>
<tr>
<td>9</td>
<td>203</td>
<td>10</td>
<td>6.6 × 10⁸</td>
<td>8.8 × 10⁷</td>
</tr>
<tr>
<td>10</td>
<td>152</td>
<td>10</td>
<td>5.6 × 10⁸</td>
<td>2.9 × 10⁷</td>
</tr>
<tr>
<td>11</td>
<td>152</td>
<td>5</td>
<td>7.6 × 10⁸</td>
<td>7.3 × 10⁷</td>
</tr>
<tr>
<td>12</td>
<td>149</td>
<td>5</td>
<td>4.2 × 10⁸</td>
<td>3.2 × 10⁷</td>
</tr>
<tr>
<td>13</td>
<td>150</td>
<td>5</td>
<td>5.6 × 10⁸</td>
<td>3.1 × 10⁷</td>
</tr>
<tr>
<td>14‡</td>
<td>236</td>
<td>5</td>
<td>4.8 × 10⁸</td>
<td>3.5 × 10⁷</td>
</tr>
<tr>
<td>15§</td>
<td>245</td>
<td>5</td>
<td>6.8 × 10⁸</td>
<td>9.8 × 10⁷</td>
</tr>
</tbody>
</table>

*Microorganisms recovered without cleaning or decontamination.
†Brushing only carried out.
‡1% Hospec used as detergent; 1% MediGene used in all other experiments.
§Flushing and brushing carried out by endoscopy Staff Nurse.
reduction achieved in three of four runs. In two of nine test runs, no cleaning was carried out and so flushing with saline for TVCs was not done.

The third stage of each test run was to re-inoculate the channels with test organisms as before, to clean them, and then to process the gastroscope in the Medivator. The channels were then flushed with saline for TVCs. In five of nine test runs, cleaning was carried out by flushing and brushing. After cleaning in this manner, followed by processing in the Medivator the counts after processing in the instrument channel were 0 cfu/ml in four runs and 7 cfu/ml in one, whilst in the air–water channel counts were 0 cfu/ml in four and two cfu/ml in one. In all five runs a $10^2$–$10^8$-fold reduction in count of the inoculum had been achieved in both channels after processing. With flushing and brushing of channels prior to processing, the initial concentration of serum present in the inoculum had no apparent effect on the reduction in count achieved after processing. In two of nine test runs cleaning was carried out by brushing alone. In these, the counts after processing ranged from 110 to $>300$ cfu/ml in the instrument channel and from $<1$ to 9 cfu/ml in the air–water channel. In both these, a $10^5$–$10^8$-fold reduction in count of the inoculum had been achieved in the air–water channel after processing but only a $10^5$–$10^6$-fold reduction in the instrument channel demonstrating the inadequacy of cleaning by brushing alone. In two of nine test runs no cleaning at all was carried out. In these, the counts after processing counts ranged from 224 to 300 cfu/ml in the instrument channel and from 0 to 33 cfu/ml in the air–water channel. In these two runs a $10^6$–$10^8$-fold reduction in count of the inoculum had been achieved in the air–water channel after processing but only a $10^5$–$10^6$-fold reduction in the instrument channel demonstrating the importance of cleaning. Little difference is apparent between the results obtained without any cleaning and those obtained with cleaning by brushing alone, which demonstrates the importance of flushing channels with detergent prior to brushing. The results also clearly show that the air–water channel is easier to decontaminate than the instrument channel.

**Second series of test runs**

In the second series of test runs (runs 10–13 in Table II) the Tristel Generator was set to generate a level of around 150 ppm of chlorine dioxide. Four tests were carried out with the actual levels of chlorine dioxide generated varying from 149 to 152 ppm. The second stage of each run was to inoculate the channels with test organisms and then to clean them prior to flushing with saline for TVCs. In all four runs, cleaning was carried out by flushing and brushing counts ranged from $1.7 \times 10^3$ to $1.0 \times 10^5$ cfu/ml in the instrument channel and from $2.7 \times 10^4$ to $2.6 \times 10^5$ cfu/ml in the air–water channel. Hence, flushing and brushing reduced the inocula in the channels $10^2$–$10^4$-fold, similar to the reductions found in the first series of test runs.

The third stage of each test run was to re-inoculate the channels with test organisms as before, to clean them, and then to process the gastroscope in the Medivator. The channels were then flushed with saline for TVCs. In all four runs, cleaning was carried out by flushing and brushing. After cleaning in this manner followed by processing in the Medivator, the counts after processing in the instrument channel ranged from 41 to 79 (average 60) cfu/ml, and in the air–water channel ranged from 3 to 55 (average 35) cfu/ml. In all four runs a $10^9$–$10^{12}$-fold reduction in count of the inoculum had been achieved in the air–water channel after-processing and a $10^5$–$10^6$-fold (three runs) or $10^6$–$10^7$-fold (one run) reduction in the instrument channel. With flushing and brushing of channels prior to processing, the initial concentration of serum present in the inoculum had no apparent effect on the reduction in count achieved after processing. As in the first series of tests, the results in the second series clearly show that the air–water channel is easier to decontaminate than the instrument channel.

Two further runs (runs 14–15 in Table II) were carried out. The first of these (run 14) was carried out to investigate the effect of using a general purpose neutral detergent (1% Hospec) rather than a dual enzymatic bactericidal detergent (1% MediGene). The level of chlorine dioxide used was 236 ppm. The results indicate that 1% Hospec was less effective than 1% MediGene particularly in cleaning the instrument channel where, after processing, 35 cfu/ml remained in the channel and the reduction achieved was $10^6$-fold rather than $10^7$–$10^8$-fold. This was repeated twice at a later date with similar results (not tabulated). In the second
run (run 15), the flushing and brushing of the inoculated gastroscope were carried out by an endoscopy staff nurse rather than by the author in order to obtain results likely to be achieved in practice, rather than in a research setting. The level of chlorine dioxide used was 245 ppm. The reductions achieved by the nurse post-processing were $10^6$ to $10^7$-fold in the instrument channel and $10^7$ to $10^8$-fold in the air–water channel. This was repeated twice at a later data with similar results (not tabulated).

Investigation of the possible bactericidal activity of detergents

Aqueous 1% solutions of MediGene and Hospec were inoculated with $10^8$ cfu/ml of *P. aeruginosa* and viable counts determined after 0, 5, 10 and 15 min. The counts obtained for MediGene were $5.2 \times 10^8$ at 0 min, $5.5 \times 10^8$ after 5 min, $5.3 \times 10^8$ after 10 min and $5.6 \times 10^8$ after 15 min whilst the counts for Hospec were $2.3 \times 10^8$ at 0 min, $1.9 \times 10^8$ after 5 min, $2.3 \times 10^8$ after 10 min and $2.0 \times 10^8$ after 15 min. Thus neither detergent exhibited any bactericidal activity over the test period.

Investigation of the possible bactericidal activity of sodium glutamate

An aqueous 10% solution of sodium glutamate was inoculated with $10^8$ cfu/ml of *P. aeruginosa* and viable counts carried out after 0, 1, 2 and 4 h. The counts obtained were $2.6 \times 10^8$ at 0 h, $3.4 \times 10^8$ after 1 h, $3.5 \times 10^8$ after 2 h and $7.2 \times 10^8$ after 4 h. Thus sodium glutamate appeared to promote bacterial growth over the test period.

Tests on a variety of endoscopes that had been used in clinical procedures

Tests were carried out on various types of endoscope including gastroscopes (10), duodenoscopes (eight) and cystoscopes (seven) following clinical procedures. These were flushed through with detergent and brushed as normal by staff in the Endoscopy theatre prior to being taken to the research laboratory. Here they were processed in the Medivator using Tristel at concentrations ranging from 145 to 245 ppm chlorine dioxide. Sterile saline was then flushed through the channels and samples inoculated on to Blood agar plates which were incubated aerobically and anaerobically. In no case were any aerobic or anaerobic micro-organisms isolated from either the instrument or air–water channels, demonstrating that endoscopes used in clinical procedures and then cleaned as normal by endoscopy staff can be reliably disinfected (and possibly sterilized) by subsequent processing in a Medivator using low concentrations of chlorine dioxide.

Discussion

An ideal agent for decontamination of flexible endoscopes has yet to be found. Such an agent would be a high-level disinfectant or sterilant, rapid in action, non-toxic and non-irritant, non-damaging to endoscope and washer/disinfector components, economical, and harmless to the environment on disposal. For many years, glutaraldehyde has been the most widely used agent for decontaminating endoscopes. However, it is far from ideal. Whilst it is a reasonably effective disinfectant which is non-damaging to endoscope and washer/disinfector components and cheap, it is toxic, irritant and allergenic. To counteract the harmful effects, an enclosed system, a fume cupboard or appropriate extraction system is required. Such systems can be very expensive and increase substantially the real cost of using glutaraldehyde. Consequently, in recent years, manufacturers have striven to develop alternatives which include chlorine dioxide formulations.

Chlorine dioxide is a relatively old disinfectant, first prepared in the early 19th century and used in the treatment of water supplies in Europe after 1850. It is a very powerful oxidizing agent with 2.5 times the oxidation capacity of chlorine. Unlike chlorine, chlorine dioxide is believed not to ionize in water, and the intact chlorine dioxide molecule is the bactericidal compound. It has been known for a long time that chlorine dioxide is a more effective sporicide than chlorine. In recent years, instrument disinfectants based on chlorine dioxide have been developed. These products are supplied as two components: a base solution (citric acid with preservatives and corrosion inhibitors) and an activator solution (sodium chlorite). Working solutions of chlorine dioxide are prepared fresh as required by mixing the two components and then diluting with water to achieve the required concentration. Chlorine dioxide products will only replace glutaraldehyde products if they prove to be superior overall in terms of microbicidal performance, toxicity and damage caused to endoscope and washer/disinfector components, and cost.
When choosing an agent/method for reprocessing endoscopes, the first consideration is microbicidal performance. The minimum standard required is high-level disinfection for endoscopes used in non-invasive procedures and sterilization, if practical, for those used in invasive procedures e.g. arthoscopes and laparoscopes. The reprocessing time should be as short as possible to facilitate a short turn-round time of instruments. The microbicidal performance of glutaraldehyde is far from ideal. Many organisms are resistant to glutaraldehyde and long exposure times are required to kill them; e.g. over 1 h for M. avium-intracellulare, over 3 h for some bacterial spores, and over 10 h for Cryptosporidium parvum cysts. The recommended sterilizing time for glutaraldehyde to reprocess endoscopes used in invasive procedures is 10 h which is impractical. Exposure times to glutaraldehyde used in endoscopy units are far shorter than is required to kill resistant organisms, which can result in contamination of endoscope washer/disinfector. The suspension tests showed that a concentration of 150 ppm chlorine dioxide rapidly destroy bacterial spores including Bacillus subtilis, resistant vegetative bacteria e.g. M. tuberculosis and M. avium-intracellulare, and other important vegetative bacteria e.g. P. aeruginosa. Also, routine use of chlorine dioxide has been found successfully to eradicate glutaraldehyde-resistant M. chelonae from the rinse water within an endoscope washer-disinfector without causing any problems associated with corrosion. Products containing chlorine dioxide levels of around 1100 ppm, designed for multi-use over 14 days, have been shown to be sporidical in 10 min and bactericidal and virucidal in 5 min when used according to the manufacturers’ prescribed conditions. In the present study, the microbicidal performance of a new low concentration, single-use chlorine dioxide product (Tristel One-Shot sterilant) was tested by means of preliminary in vitro suspension tests followed by practical trials in a Medivator washer/disinfector. The suspension tests showed that a concentration of ≤150 ppm chlorine dioxide was rapidly microbicidal for S. aureus and B. subtilis under simulated dirty conditions. Two series of tests were carried out in the Medivator washer/disinfector using chlorine dioxide. In the first, with 201–235 ppm, cleaning followed by processing in the Medivator consistently achieved a >10^4-fold reduction of test organisms, and the second, with 149–152 ppm, consistently achieved a >10^5-fold reduction of test organisms.

At present, there is no standard test method for assessing sterilants for flexible endoscopes and no official performance level which must be achieved. European standards for disinfectants require a ≥10^5-fold reduction of test bacteria. In the absence of a standard for assessing sterilants for endoscopes, it is debated whether a reduction of ≥10^5-fold is required. In the present study it was found that low levels of chlorine dioxide can achieve either level of performance. It is concluded that, in terms of microbicidal performance, chlorine dioxide is far superior to glutaraldehyde and offers the prospect of rapid high-level disinfection and, possibly, sterilization of endoscopes.

Toxicity is also important when choosing an agent/method for reprocessing endoscopes. In endoscopy units using glutaraldehyde, health surveillance of staff is mandatory and should include a pre-employment enquiry regarding asthma, skin and mucosal sensitivity problems and lung function tests. Alternatives to glutaraldehyde need to be significantly less toxic. In this respect, chlorine dioxide is not an ideal alternative. Although described by the manufacturer as user-safe, unpleasant irritating fumes are given off during preparation and use. Consequently, it is strongly recommended that vapour emissions are extracted and/or suitably contained. The amount of fumes given off by chlorine dioxide formulations increase with the concentration of chlorine dioxide. One of the purposes of developing Tristel One-Shot sterilant was significantly to reduce the amount of fumes. In the UK, the Health and Safety Executive has set an OES for chlorine dioxide: the short-term exposure limit (15 min reference period) is 0.3 ppm whilst the long-term exposure limit (8 h time weighted average reference period) is 0.1 ppm. In comparison, because no safe exposure limit can be identified for glutaraldehyde, the Health and Safety Executive has set a MEL of just 0.05 ppm (short-term and long-term exposure limit) and advised that ‘wherever possible, glutaraldehyde should be replaced by a less hazardous substance’. It is concluded that whilst chlorine dioxide is not user-friendly it is much safer to use than glutaraldehyde and the use of low concentration formulations such as Tristel One-Shot sterilant will reduce vapour emissions to a minimum.

The potential for damaging equipment also requires consideration. Glutaraldehyde causes little, if any, damage. The only real problem encountered is channel blockage. Glutaraldehyde is a fixative.
Consequently, if channels are not cleaned thoroughly after every procedure then layers of fixed material build up until blockage occurs. Most new sterilants marketed as alternatives to glutaraldehyde are not fixatives but are strong oxidizing agents e.g. peracetic acid, hydrogen peroxide, chlorine dioxide and ‘superoxidized’ water. Consequently, the possibility of damage to endoscopes and washer/disinfectors is a major concern to endoscopy departments that are contemplating a switch from glutaraldehyde to one of these new sterilants. Experience has shown that long-term use of products containing high concentrations of chlorine dioxide can result in damage to the outer plastic coat of the insertion tube of a flexible endoscope.

The risk of damage to endoscopes and washer/disinfectors can be minimised in many ways. Firstly, corrosion inhibitors and buffers can be incorporated into chlorine dioxide sterilants. Secondly, the contact time between sterilant and endoscopes and washer/disinfectors can be reduced to a minimum. Thirdly, after disinfection/sterilization, endoscopes and washer/disinfectors can be rinsed thoroughly with water of suitable microbiological quality.  

Fourthly, manufacturers can develop single-use products which lack the high concentrations needed by multi-use formulations to allow for the effects of dilution during repeated use. Fifthly, a protective coating resistant to oxidizing agents e.g. of urethane–silicone copolymer can be applied to the outer surface of the insertion tube. Sixthly, an anti-oxidizing agent can be added to the final rinse water to neutralize any residues of sterilant on the endoscope.

Overall, it is concluded that chlorine dioxide is far superior to glutaraldehyde in terms of spectrum and speed of micobical performance and toxicity, but inferior in the risk of damage to endoscopes and washer/disinfectors. Glutaraldehyde is a cheaper sterilant but savings may be negated by the cost of mandatory containment systems and health surveillance of staff.

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