**Mycobactericidal activity of chlorine dioxide wipes in a modified prEN 14563 test**

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**KEYWORDS**
Mycobactericidal activity
Disinfectant
Chlorine dioxide; Chlorine

**Summary**
Tristel Sporicidal Wipes are chlorine dioxide-based disinfectant wipes for disinfecting non-lumened semi-critical medical devices. In this study, the mycobactericidal activity of this product was assessed by a modified version of the European Standard prEN 14563 carrier test under clean conditions against *Mycobacterium avium*. The chlorine dioxide concentration in the activated wipe was 200 ppm. The results showed that the chlorine dioxide wipes were mycobactericidal in 30 s contact time with mechanical action and in 60 s without mechanical action, both under clean conditions.

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**Introduction**
For many years, glutaraldehyde was the most widely used disinfectant for decontaminating flexible endoscopes and other medical devices. It provides a resilient, wide spectrum of microbicidal action and is therefore considered a high-level disinfectant for heat-labile medical equipment. However, several researchers have questioned its mycobactericidal activity due to the inability of glutaraldehyde to rapidly penetrate the waxy layers which make up mycobacterial cell walls. Allergic contact dermatitis, asthma, rhinitis and epistaxis have been observed in healthcare workers exposed to glutaraldehyde, since its vapours irritate eyes, nose and throat at environmental concentrations of 0.2 ppm. Moreover, continuous use of 2% glutaraldehyde in endoscope washer-disinfector machines has selected resistant strains of several micro-organisms to this disinfectant, such *Mycobacterium chelonae*. For these reasons among others, multiple studies have been carried out to assess less
toxic or irritating alternatives, used either manually or in washer-disinfector machines. Peracetic acid, ortho-phthalaldehyde, superoxidised water, gas plasma and chlorine dioxide have been introduced as suitable alternatives to glutaraldehyde for endoscopes and other heat-sensitive medical devices.\textsuperscript{6,10} Furthermore, Tristel Sporicidal Wipes\textsuperscript{15} are a ready-to-use product. The disinfectant was freshly prepared according to the manufacturer’s instructions and used immediately (within 5 min). The Tristel Sporicidal Wipe system has two components: a wipe which is saturated with a mixture of organic acids, preservatives, buffers and corrosion inhibitors, and a bottle containing a sodium chlorite-based foam. Prior to testing, the wipes were prepared by squirting foam onto the wipe and then scrunching it by hand to mix the two components of the product to activate the disinfectant.

Sterile distilled water was used as the disinfectant control.

\textbf{Mycobacterial suspensions}

\textit{Mycobacterium avium} American Type Culture Collection 15769 was used as the test organism. Stock cultures were stored at $-80\,\text{C}$ and prior to testing were thawed and spread onto 7H11 Middlebrook plates with oleic acid albumin dextrose catalase (OADC) supplement (bioMérieux, Boxtel, The Netherlands) and incubated for 21 days at 37\,\text{C}.

Test suspensions were prepared by suspending the harvested mycobacteria in diluent (tryptone sodium chloride solution) and homogenising them with sterile glass beads for 5 min. Ten millilitres of distilled water were added, stirred and the suspension left to settle for 20 min. This supernatant fluid was adjusted by spectrophotometer absorbance to obtain a concentration of $10^7$ to $10^9\,\text{cfu/mL}$. The test suspension was enumerated by performing 10-fold dilutions in diluent up to $10^{-6}$ to $10^{-8}$, and then cultured onto 7H11 Middlebrook plates with OADC supplement (M7H11). Plates were incubated at $37\,\pm\,1\,\text{C}$ for 21 days. One millilitre of each suspension dilution was plated onto 7H11 agar in duplicate.

The disinfectant activity was tested with an organic load by preparing test suspensions in 0.3 g/L bovine serum albumin (BSA) (i.e. clean conditions). Freshly prepared test suspensions with organic matter were used as initial inocula for all tests and used in a 2 h time period.

\textbf{Quantitative carrier test}

The carrier test was performed using a modified technique described by the European Standard prEN 14563. In brief, sterile frosted glass was used as germ carrier on which aliquots of 50 \(\mu\text{L}\) of each test suspension with organic load were applied and left to dry at 36\,\pm\,1\,\text{C}. The contaminated area was then covered with chlorine dioxide-moistened wipes and disinfectant action assessed with and without mechanical action. The mechanical action was achieved by rubbing the wipe for 3 s every 10 s
on the contaminated carrier area. Immediately after contact times of 30 s and 1 min, the carriers were inserted in tubes containing 10 mL of neutralising agent at 20°C using sterile forceps. The tubes were vortexed for 15 s with glass beads to elute the mycobacteria from the carrier surface. After 5 min neutralisation time, the eluates were serially diluted up to $10^{-3}$ in neutraliser, and 1 mL of the mixture spread over M7H11 agar medium plates in duplicate and incubated as described in the mycobacterial suspension. The counts from these were designated 'Na' with mechanical action or 'Na1' without mechanical action.

Residues of the remaining disinfectant were neutralised using the dilution—neutralisation method. The efficacy of the neutraliser was assessed, as described in the European Standard draft. Sodium chlorite and a reactant were mixed in equal volumes to produce 200 ppm of chlorine dioxide. A neutralisation control was also performed placing an inoculated dried carrier in contact with the neutralised disinfectant and processed as mentioned above. The neutraliser contained 3% (v/v) Tween 80, 0.3 (w/v) lecithin, 3% (v/v) saponin and 0.4 (w/v) sodium lauryl sulphate in saline solution. The non-toxicity of neutraliser was ensured in additional trials.

In addition to water controls required in prEN 14563 (Nw1), other specific water controls were included. Each carrier test was covered with wipes soaked in water instead of disinfectant. Two of the contaminated carriers were tested with wipes soaked in 3 mL of distilled water, one with mechanical action (Nw2) and another without mechanical action (Nw3). These water controls were carried out at room temperature. Distilled water was used for the water controls because the Tristel Sporicidal Wipe is a ready-to-use product. The procedure was also valid under experimental conditions and method validation for ready-to-use products.

**Interpretation of results**

In the prEN 14563 test the disinfectant efficacy was determined by comparing growths on the control and disinfectant plates and reported as the log$_{10}$ reduction in the number of cfu/carrier at each contact time ($\log R = \log Nw - \log Na$). Disinfectants are considered mycobactericidal when reduction by a factor of at least 10$^4$ in the initial inoculum has been demonstrated.

**Results**

Test suspension controls showed bacterial concentrations with the initial inoculum of $2.13 \times 10^9$ cfu/mL. The dilution—neutralisation method and neutraliser were found to be effective and to result in no inhibition of mycobacterial growth (data not shown). Controls of test suspensions, dilution—neutralisation method and the recovery of bacteria from carriers were satisfactory and confirmed the results obtained (data not shown).

Data obtained in the water controls with and without wiping (i.e. with and without mechanical action) are presented in Table I. The data in Table II show the efficacies of the disinfectant against mycobacteria. Tristel Sporicidal Wipes showed reduction by a factor of $10^4$ against $M. avium$ in 30 and 60 s with and without mechanical action, respectively.

**Discussion**

As draft European Standards are now available for mycobactericidal and tuberculocidal efficacy of chemical products for medical instruments, disinfection validation can be performed. A disinfectant for medical devices is deemed suitable for killing mycobacteria if it passes the prEN 14563 (carrier test) standard. The findings of this study suggest that the Tristel Sporicidal Wipes have mycobactericidal activity when following the manufacturer’s instructions for use. They were proven to be effective against $M. avium$ within 30 s contact time using mechanical action at the activated concentration (200 ppm) under clean conditions as specified in the prEN 14563. Additionally, the product itself without mechanical action has proven to be mycobactericidal within in 60 s.

The only conditions and provisions to demonstrate bactericidal efficacy in vitro applicable to disinfectants impregnated in wipes are laid out in EN 1276 suspension test for institutional and food areas. Nevertheless, the procedure includes no particular specification for products of this nature. On the other hand, the possibility of assessing

**Table I** Water controls: standard test and product adaptation water control with wipe and with or without mechanical action

<table>
<thead>
<tr>
<th>Water control</th>
<th>Xwm</th>
<th>log Nw</th>
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<tbody>
<tr>
<td>Nw1</td>
<td>$4.57 \times 10^7$</td>
<td>7.65</td>
</tr>
<tr>
<td>Nw2</td>
<td>$7.48 \times 10^6$</td>
<td>6.87</td>
</tr>
<tr>
<td>Nw3</td>
<td>$2.4 \times 10^7$</td>
<td>7.38</td>
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</tbody>
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Nw1: water control (normative assay).
Nw2: water control with wipe and mechanical action.
Nw3: water control with wipe but without mechanical action.
Xwm: weighted mean of count per mL.
disinfectant products in wipe format for the medical field has never been posed and, as such, no suggestion has been made about applying any biocidal activity regulation. The carrier test was chosen here because bacteria are dried on a surface and are less accessible to biocides than those in homogeneous suspension. Moreover, the test conditions are closer to real-life conditions, since the carrier can be rubbed with the wipe.

In addition to the water control as described in the prEN 14563 validation procedure, we included controls with water-impregnated wipes with and without mechanical action (rubbing) on the carrier. As expected, the mycobacteria count after mechanical action was slightly less, but proved that mechanical action alone was insufficient to eliminate \( M. \text{avium} \) from the carrier.

Effective neutralisation in testing disinfectant solutions is considered a crucial element to ensure test result validity. Neutralisation must be effective to prevent any residual bacteriostatic or bactericidal activity, yet at the same time it must be non-toxic to the test organism. This is particularly important for short application times such as 30–60 s.

\( M. \text{avium} \) is an environmental, opportunistic human pathogen which infects advanced-stage acquired immunodeficiency syndrome patients, and is known to be resistant to disinfectants such as quaternary ammonium compounds, phenols, glutaraldehyde and chlorine.\(^{10,17}\) The main problem with in-vitro studies of mycobacterium species, including \( M. \text{avium} \), is that it forms aggregates or clumps during the suspension procedure and, if these are not excluded, can lead to spurious disinfection resistance and variable colony counts due to irregular dispersal of aggregates. Other authors have shown that growth conditions, metabolic state of the cells, and colony type influence mycobacterial susceptibility to disinfectants.\(^{10}\) These problems have currently been reduced through the use of standardised procedures such as those in prEN 14536.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Mycobactericidal activity of Tristel Sporicidal Wipes</th>
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<tbody>
<tr>
<td></td>
<td>With mechanical action</td>
</tr>
<tr>
<td></td>
<td>( \log \text{Na} )</td>
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<tr>
<td>30 s</td>
<td>2.76</td>
</tr>
<tr>
<td>60 s</td>
<td>2.38</td>
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</tbody>
</table>

\( \text{R: reduction} (\log \text{R} = \log \text{Nw2} - \log \text{Na}) \)

\( \text{R1: reduction} (\log \text{R1} = \log \text{Nw3} - \log \text{Na1}) \)

\( \text{Na and Na1: mycobactericidal action.} \)

There have been few studies published on in-vitro testing of mycobactericidal activity, hence comparison was difficult to ascertain due to differences in methodology, especially protocols and concentration of products used, and the strain of micro-organism. This work seems to be the only study where the chlorine dioxide mycobactericidal activity has been assessed in vitro on a carrier test; and is likewise unique in using the new European Standards to do so. In our work, 200 ppm of chlorine dioxide at 30 and 60 s of contact time showed reduction by a factor of \( 10^6 \). This criterion is the minimum requirement for passing the quantitative carrier test. Some authors also found a mycobactericidal activity, but in suspension tests, at high (600–1100 ppm)\(^{12,18}\) and low (30 ppm)\(^{16}\) concentrations, against \( M. \text{avium-M. intracellulare} \) (clinical strains) at 60 s of contact time. The presence of BSA (0.3 g/L, final concentration) affected the mycobactericidal activity of 30 ppm chlorine dioxide. The study presented here only assessed the mycobactericidal activity under clean conditions, i.e. BSA of 0.3 g/L in the final concentration, and did not assess the disinfectant’s activity under dirty conditions (3 g/L of BSA plus 3 mL/L sheep erythrocytes). Clean conditions were selected as the appropriate organic challenge as the Tristel Sporicidal Wipes are clearly labelled for use only after the instrument or surface has been pre-cleaned prior to disinfection.

Several studies of flexible endoscope disinfection have demonstrated that chlorine dioxide is effective against different micro-organisms, including mycobacteria.\(^{13,18}\) Although concentrations of 150–250 ppm of chlorine dioxide are commonly used for endoscope disinfection, the use of chlorine dioxide wipes has been very limited.\(^{13}\) Some authors found that chlorine dioxide wipes are a satisfactory alternative means for the disinfection of flexible pharyngolaryngoscopes, rigid nasendoscopes and ultrasound transducer heads.\(^{14,19,20}\) Nevertheless, further in-use studies are required to prove these preliminary findings.

In conclusion, Tristel Sporicidal Wipes showed rapid efficacy against \( M. \text{avium} \) in a quantitative carrier test in clean conditions at a concentration of 200 ppm chlorine dioxide.

**Conflict of interest statement**

None declared.

**Funding sources**

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