Sporicidal activity of Superoxidised water

Hospital Infection Research Laboratory
City Hospital NHS Trust
Dudley road Birmingham, B18 7QH,
April 1999

C.R. Bradley, Dr. A.P.Fraise and J.R. Babb

SPONSOR
Aquastel UK Ltd, 7 Dunfermline Business Centre, Izatt Avenue, Dunfermline, Fife, KY11 3BZ

EQUIPMENT PROVIDED BY
Aquastel ATU Ltd /Ass-Tec Ltd, Eildon Factory, Tweedbank, Galashiels, TD1 3RP

DISINFECTANT TESTED
Superoxidised water (Anolyte) produced on a Eurostel (EVOI) unit with following production criteria:
- ORP: >1100 mV
- pH: 2 - 3.5
- C.ac: < 50 mg/l
- Current: 4 amp
- Flow rate: 60 LPH
- Salt concentration: 26% (saturated solution)

OBJECTIVE
To test the sporicidal activity of Superoxidised water using a suspension test in the absence and presence of an additional organic load.

For Comparative purposes, tests were also carried out with 2% activated alkaline glutaraldehyde (Asep, Galen Ltd, Craigavon, Northern Ireland). 2% glutaraldehyde is, at present, recognised as the disinfectant of choice for flexible fibreobtic endoscopes and other heat sensitive instruments but it is irritant and sensitiser and a safer alternative is sought.

TEST METHODS
European (CEN) phase 2 tests to establish sporicidal activity have not yet been agreed. The suspension tests used here has been widely used to test instruments and was first described by Babb JR, Bradley CR, Ayliffe GAJ (1980). Sporicidal activity of glutaraldehydes and other factors influencing their selection for the treatment of medical equipment. Journal of Hospital Infection 1: 63-75.

Recovery/Neutraliser broth
Nutrient broth containing 1 % sodium thiosulphate used for superoxidised water.

Nutrient broth (Oxoid No. 2) prepared at double strength with the addition of 10% horse serum after sterilisation. These media have been shown as suitable in recovering small numbers of test organism in the presence of the disinfectants under test. They are not inhibitory but neutralise disinfectant residues carried over to the recovery system.

**SPORICIDAL ACTIVITY**

**Test organism**

Bacillus subtilis var Niger NCTC 10073 spores (UK chemical sterilisation validation test strain)

**Suspension test**

A suspension of Bacillus subtilis var niger, was heat shocked (80 C for 1 min) to eliminate non sporing organisms (>10⁷/ml) and 1 ml to 9 ml of freshly prepared disinfectant. The mixture was gently swirled to mix and, at specific time intervals of 1,2,5,10,20,30,60 and 120 min, 1 ml was added to 9 ml of recovery/neutraliser broth. This was mixed thoroughly and 10 fold diluted in quarter strength Ringers solution. The recovery broth and dilutions were plated onto tryptone soya agar plates, incubated for 18 hours at 37 C and examined for surviving test organisms.

The test was also performed in the presence of horse serum to simulate dirty (in use) conditions. 10% Horse serum was added to the spore suspension to give a final concentration in the test spore/disinfectant mixture of 1% serum.

Surviving test organisms were enumerated and survivors transported to log_{10} counts. The recovery broths were incubated for a 7 days at 37 C to give damaged spores the opportunity to germinate before plating out onto tryptone soya agar to confirm their identity.

The pH and oxidation reduction potential (ORPI) of the solution were measured immediately after generation and at the end of the test period of 2 hours.

**RESULTS**

The sporicidal activity of Superoxidised water and 2% activated alkaline glutaraldehyde under clean conditions is shown in table 1. In the absence of additional organic load, superoxidised water achieved a 6 log_{10} reduction in 5 min. However, in the presence of 1 % serum, Superoxidised water had no demonstrable sporicidal activity over 2 hours. In comparison, 2% glutaraldehyde was less rapidly effective as a sporicidal agent i.e. 1-2 hours to achieve a 6 Log_{10} reduction but, unlike the Superoxidised water activity was not impaired with the addition of 1% serum as an organic load.
### TABLE 1

Log$_{10}$ spores remaining after exposure to Superoxidised water or 2% glutaraldehyde

<table>
<thead>
<tr>
<th>Contact time</th>
<th>Superoxidised water</th>
<th>2% glutaraldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clean conditions</td>
<td>Dirty conditions</td>
</tr>
<tr>
<td>Pre disinfecting challenge</td>
<td>7.76</td>
<td>7.74</td>
</tr>
<tr>
<td>1 min</td>
<td>4.84</td>
<td>7.61</td>
</tr>
<tr>
<td>2 min</td>
<td>2.34</td>
<td>7.60</td>
</tr>
<tr>
<td>5 min</td>
<td>1.30</td>
<td>7.58</td>
</tr>
<tr>
<td>10 min</td>
<td>0</td>
<td>7.60</td>
</tr>
<tr>
<td>20 min</td>
<td>0</td>
<td>7.58</td>
</tr>
<tr>
<td>30 min</td>
<td>0</td>
<td>7.44</td>
</tr>
<tr>
<td>1 hour</td>
<td>0</td>
<td>7.46</td>
</tr>
<tr>
<td>2 hours</td>
<td>0</td>
<td>7.39</td>
</tr>
</tbody>
</table>

The production parameters recorded were:

- **pH:** 2.85 (start) 3.59 (end)
- **ORP:** 1152 mV (start) 1150 mV (end)

### CONCLUSION

This study shows that Superoxidised water (ORP >1100 mV, pH 2.0-3.5 and C.ac <50mg/l) generated using the prototype equipment produced by Aquastel ATU /Ass-tec Ltd was highly effective as a sporicidal agent in the absence of additional organic material.

A 6 log$_{10}$ reduction in test spores was achieved with freshly generated solution in 5 minutes. This is far more rapid than the widely used 2% glutaraldehyde.

However, when 1% horse serum was added as an organic load no appreciable sporicidal activity was noted in 2 hours. Items must therefore be scrupulously clean before they are disinfected using this agent. Initial cleansing using validated automated system is advised.

Further tests are recommended to establish the sporicidal activity of aged solutions and the microbactericidal activity of the Superoxidised water. Testing with Mycobacterium tuberculosis or Mycobacterium terrae is advised to establish tubercological (high level) disinfectant activity.

If these and in vivo tests (i.e. in a washer disinfector) show that the agent is effective, safe, non damaging and affordable it may prove a worthy alternative to 2% glutaraldehyde for disinfecting heat flexible endoscopes (see Babb and Bradley 1995). The generator (prototype) used in this study would be unsuitable for use with an automated system as the volume (60LPH) is too low.
References


Birmingham, April 1999

Signed:

JR Babb
Laboratory Manager

CR Bradley
Senior MLSO

Dr. AP Fraise
Director