

Sporicidal activity of Superoxidised water

Hospital Infection Research Laboratory
City Hospital NHS Trust
Dudley road Birmingham, B18 7QH,
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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 fibreoptic 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 ($>10^7$ /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 log₁₀ 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 log₁₀ 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 Log₁₀ reduction but, unlike the Superoxidised water activity was not impaired with the addition of 1% serum as an organic load.

TABLE 1**Log₁₀ spores remaining after exposure to Superoxidised water or 2% glutaraldehyde**

Contact time	Superoxidised water		2% glutaraldehyde	
	Clean conditions	Dirty conditions	Clean conditions	Dirty conditions
Pre disinfecting challenge	7.76	7.74	7.76	7.74
1 min	4.84	7.61	7.63	7.59
2 min	2.34	7.60	7.60	7.56
5 min	1.30	7.58	7.46	7.36
10 min	0	7.60	7.19	7.24
20 min	0	7.58	6.87	7.74
30 min	0	7.44	6.34	6.48
1 hour	0	7.46	2.75	2.72
2 hours	0	7.39	0	0

The production parameters recorded were:

pH: 2.85 (start) 3.59 (end)
ORP: 1152 mV (start) 1150mV (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 log₁₀ 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 disinfectant) 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

Babb JR, Bradley CR & Ayliffe GAJ. Sporicidal activity of glutaraldehyde and hypochlorites and other factors influencing their selection for the treatment of medical equipment. *Journal of Hospital Infection* (1980) 1: 63-75.

Babb JR and Bradley CR. A review of glutaraldehyde alternatives. *British Journal of Theatre Nursing* (1995) 5 (No7): 20-24.

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Signed:

JR Babb
Laboratory Manager

CR Bradley
Senior MLSO

Dr. AP Fraise
Director