

# **Tuberculocidal 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

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## **EQUIPMENT PROVIDED BY**

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## **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 assess the tuberculocidal activity of superoxidised water (generated using the Aquastel/Ass-Tec machine) using a suspension test in the presence and absence of 1% serum as an organic load.

## **MATERIALS AND METHODS**

Test method used is that of Griffiths et al. Journal of Hospital Infection (1998) 38, 183-192

### **Disinfectant**

Superoxidised water was freshly generated on site and tested immediately after generation.

### **Test Organism**

Mycobacterium tuberculosis H37 Rv NCTC 7416 was used as the test organism

The type strain of *Mycobacterium tuberculosis* H37 Rv was obtained freeze, dried from the National Collection of Type Cultures, Central Public Health laboratory, Colindale, London. The glass vial was carefully broken and an aliquot of 7H9 broth was added to the tube to rehydrate the organism. One hundred  $\mu\text{l}$  of this suspension was spread on Middlebrook 7H11 agar with OADC supplement (Becton Dickinson Ltd.) and incubated at 37°C for 21 days.

One colony of the test organism was taken from the plate after 21 days incubation and inoculated into 100ml 7H9 broth and incubated at 37°C for 21 days. The suspension was subjected to ultrasonics (50 Hz) for 10 minutes every second day and inverted several times to minimise clumping. Ten per cent glycerol was added as a preservative and this also helped to maintain a homogeneous suspension. One n-fl aliquots of the suspension were decanted into 1.5ml microcentrifuge tubes and held at -70°C until required.

### **Neutralisation and Recovery**

A combination of dilution and 0.5% Tween 80 was used to neutralise the disinfectant. Tests were carried out to establish the suitability of this medium in disinfectant residues carried over to the recovery system without a growth inhibiting effect. Middlebrook 7H11 agar plates were used to recover surviving bacteria.

### **Preparation of Test Suspension**

Prior to testing, one of the suspensions was removed from the freezer, thawed at room temperature, centrifuged, washed twice and spread onto a 7H11 plate for confluent growth using a sterile swab. One loopful was also spread on a 7H 11 plate for single colonial to confirm purity of the suspension. After 21 days incubation at 37°C, the growth was harvested from the plate and mixed with moistened (sterile distilled water) glass beads in a sterile bottle for five minutes. Ten ml of sterile distilled water was added to the beads, shaken and the mixture left to settle for 30 minutes. The supernatant was removed to a second sterile bottle and left to settle, by gravity, for a further 2 hours. The supernatant from this suspension was used as the test suspension for disinfectant tests.

### **Suspension Test**

One hundred  $\mu\text{l}$  of the test suspension was added to 900 $\mu\text{l}$  of the disinfectant in a microcentrifuge tube. After contact time of 5, 10, 20 and 30 minutes, 10 $\mu\text{l}$  were removed and added to 990 $\mu\text{l}$  of Ringers and Tween neutralisation/recovery medium. This was then serially diluted to  $\mu\text{l}$  in Ringers solution. One hundred  $\mu\text{l}$  of the neat and subsequent dilutions were spread onto 7H11 agar in duplicate using sterile spreaders. Plates were incubated at 37°C and checked for growth after 2 weeks incubation and thereafter every week for up to 6 weeks.

NB- all tests were carried out in

## RESULTS

The tuberculocidal activity of Superoxidised water under clean and dirty conditions is shown in table 1.

Contact time	Mean log <sub>10</sub> reductions obtained	
	Clean conditions	Dirty conditions
Pre disinfection count (challenge)	9.24	9.24
1 min	1.56	0
2 mins	2.88	0.13
5 mins	>6.24	0.17
10 mins	>6.24	0.15
20 mins	>6.24	0.09
30 mins	>6.24	0.09

A > 5log<sub>10</sub> reduction in Mycobacterium tuberculosis is indicative of tuberculocidal activity i.e. high level disinfection. This was achieved under clean conditions (absence of organic matter) in 5 minutes.

Little or no reduction in test mycobacteria was detected in 30 mins when Superoxidised water was tested in the presence of 1% serum (dirty conditions).

## CONCLUSION

From the results obtained in these tests, it would appear that Superoxidised water generated using the Aquastel/Ass-Tec equipment is tuberculocidal in 5 minutes under clean conditions.

However, there is little or no tuberculocidal activity over a 30 minute period when tested under dirty conditions ie. In the presence of 1% serum. At least a 5Log<sub>10</sub> reduction (99.999%) is necessary to meet European test criteria. It appears the efficacy of the Superoxidised water is adversely affected by the presence of organic matter. It could only be recommended as an intermediate/high level disinfectant if items are scrupulously cleaned prior to exposure to the disinfectant. This is likely to be achieved if it is used once only for the disinfection of heat labile endoscopes following thorough cleaning using an effective automated endoscope washer disinfectant (e.g. Plade Ltd).

For comparative data on the tuberculocidal activity of 2% glutaraldehyde and an super oxidised water see the article In the Journal of Hospital Infection (1999) 41 (59-70) by Selkon, Babb and Morris.

## 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