

**MICROBICIDAL ACTIVITY OF  
STERICHELE S-2000**

**FOR**

**PEARSON and BLACK Ltd**

Hospital Infection Research Laboratory

City Hospital NHS Trust

Dudley Road

Birmingham B18 7QH

**SPONSOR: Pearson and Black Ltd. Gold Crest, Guisborough, Cleveland. TS 14 8 PJ.**

**DISINFECTANT TESTED: Sterichelle S-2000** - A mixture of ammonium chloride compounds.

Concentration tested : 1 part Sterichelle to 63 parts standard hardwater

**OBJECTIVES:**

- 1. To determine the bactericidal activity under clean and dirty conditions using *Pseudomonas aeruginosa* and *Staphylococcus aureus* as test organisms**
- 2. To determine the mycobactericidal activity under clean and dirty conditions using *Mycobacterium terrae* (proposed CEN surrogate for *Mycobacterium tuberculosis*)**
- 3. To determine the sporicidal activity under clean and dirty conditions using *Bacillus subtilis var niger* spores.**

**TEST METHODS**

**Recovery/Neutralizer broth**

Nutrient broth (Oxoid No. 2) containing Leithin, Tween 80 and sodium lauryl sulphate.

This recovery medium has been shown as suitable in recovering small numbers of test organisms in the presence of the disinfectant under test. It is not inhibitory but neutralizes disinfectant residues carried over to the recovery system.

**BACTERICIDAL ACTIVITY**

**Suspension Test: clean and dirty conditions**

The test method used is based on the draft European Standard prepared by Working Group 1 (WG1) of CEN Technical Committee 216 as the phase 2/step 1 test. The method is a suspension test for disinfectants that are to be used for medical purposes. Activity of the use concentration against 'type collection' test bacteria was determined at 1, 5, 10, 20 and 30 minutes in the absence and presence of an organic load. To satisfy the requirements of the test, a 5 log<sub>10</sub> reduction in test organisms is required within 5 mins.

<b>Test organisms</b>	<i>Pseudomonas aeruginosa</i>	NCTC 6749
	<i>Staphylococcus aureus</i>	NCTC 4163

The test suspensions were prepared by removing growth from the surface of tryptone soya agar plates into 10 ml Ringers solution containing sterile glass beads. Suspensions were mixed thoroughly and allowed to stand for 5 mins before use. One ml of each test suspension was added to 9 ml of freshly prepared disinfectant. The mixtures were gently swirled to mix and, at specific time intervals of 1, 5, 10, 20 and 30 minutes, 1 ml was removed and added to 9 ml of recovery/neutralizer broth. These samples were 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<sup>0</sup>C and examined for surviving test organisms.

The number of colony forming units (cfu's) of surviving test organisms were enumerated and the results transposed to log<sub>10</sub> counts. The test was also performed in the presence of 10% horse serum (dirty conditions). This gives a final concentration of 1% horse serum in the disinfectant/spore mixture.

Efficacy was established as the mean log 10 reduction ie  $\text{Log}_{10} \text{reduction pre disinfection} - \text{Log}_{10} \text{reduction post disinfection} = \text{Log}_{10} \text{reduction}$

## **SPORICIDAL ACTIVITY**

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

**Test organism** *Bacillus subtilis var niger* NCTC 10073 (UK chemical sterilization validation test strain).

### **Suspension test: clean and dirty conditions**

Two ml of a suspension of *Bacillus subtilis var niger* containing > 10<sup>7</sup> viable spores/ml, was heat shocked (80<sup>0</sup>C for 1 mm) to eliminate non sporing organisms and added to 18 ml of freshly prepared disinfectant. The mixture was gently swirled to mix and, at specific time intervals of 1, 5, 10, 20, 30 mm, 1, 2, 3, 4, 5 and 6 hours, 1 ml was removed and added to 9 ml of recovery/neutralizer 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<sup>0</sup>C and examined for surviving test organisms. The number of viable spores in the heat shocked spore suspension (challenge) was also determined using this technique.

The number of colony forming units (cfu's) of surviving test organisms were enumerated and the results expressed as log<sub>10</sub> counts. The recovery broths were incubated for a further 7 days at 37<sup>0</sup>C, to give damaged spores the opportunity to germinate, before plating out onto tryptone soya agar to confirm their identity. The test was also performed in the presence of 10% horse serum (dirty conditions). This gives a final concentration of 1% horse serum in the disinfectant/spore mixture.

#### **MYCOBACTERICIDAL ACTIVITY**

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

**Test Organism**      *Mycobacterium terrae* NCTC 10856 (now accepted as surrogate for Myco. tuberculosis by CEN)

The type strain of *Mycobacterium terrae* NCTC 10856 was obtained freeze dried from the National Collection of Type Cultures, Central Public Health laboratory, Colindale, London. The glass vial was carefully broken and a small aliquot of 7H9 broth was added to the tube to rehydrate the organism. One hundred µl of this suspension was spread on Middlebrook 7H11 agar with OADC supplement (Becton Dickinson Ltd.) and incubated at 37<sup>0</sup>C for 14 days.

One colony of the test organism was taken from the plate after 14 days incubation and inoculated into 100ml 7H9 broth and incubated at 37<sup>0</sup>C for 14 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 ml aliquots of the suspension were decanted into 1.5ml microcentrifuge tubes and held at -70<sup>0</sup>C until required.

### 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 7H11 plate for single colonies to confirm purity of the suspension. After 14 days incubation at 37<sup>0</sup>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 then subjected to ultrasonics at 50-60 Hz for 10 mins and was then used for the suspension tests.

### Suspension Test: clean and dirty conditions

One hundred µl of the test suspension was added to 900µl of the disinfectant in a microcentrifuge tube. After contact times of 1, 5 and 10 minutes, 10 µl were removed and added to 990µl of Ringers and Tween neutralization/recovery medium. This was then serially diluted to 10<sup>-3</sup> in Ringers solution. One hundred µl of the neat and subsequent dilutions were spread onto 7H11 agar in duplicate using sterile spreaders. Plates were incubated at 37<sup>0</sup>C and checked for growth after 14 days incubation.

### RESULTS:

Table 1

### Bactericidal activity of Sterichelle S-2000

Contact time	Log <sub>10</sub> reductions achieved			
	<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>	
	Clean conditions	Dirty conditions	Clean conditions	Dirty conditions
Log <sub>10</sub> Pre count	7.53	7.53	7.60	7.60
1 mm	>5.53	>5.53	>5.60	>5.60
5 mins	>5.53	>5.53	>5.60	>5.60
10 mins	>5.53	>5.53	>5.60	>5.60
20 mins	>5.53	>5.53	>5.60	>5.60
30 mins	>5.53	>5.53	>5.60	>5.60

Table 2

**Mycobactericidal activity of Sterichelle S-2000**

**Test organism : Mycobacterium terrae NCTC 10856**

Contact time	Log <sub>50</sub> reductions in Mycobacterium terrae	
	Clean conditions	Dirty Conditions
Log <sub>10</sub> Pre count	9.49	9.44
1 mm	0.27	0.17
5 mins	0.59	0.34
10mins	1.06	0.72
20mins	1.22	0.75
30mins	1.38	0.97
60mins	1.46	1.15

Table 3

**Sporicidal activity of Sterichelle S-2000**

**Test organism *Bacillus subtilis var niger***

Contact time	Log <sub>10</sub> reductions in Bacillus subtilis spores	
	Clean conditions	Dirty Conditions
Log <sub>10</sub> Pre count	7.75	7.43
5 mins	0.51	0.39
10mins	0.55	0.15
20 mins	0.68	0.55
30mins	0.88	0.75
1 hour	0.99	0.62
2 hours	1.55	0.71
3 hours	2.28	1.70
4 hours	2.85	1.72
6 hours	5.34	3.94

**CONCLUSION**

Sterichelle S-2000, at a use dilution of 1 part to 63 parts standard hard water, was rapidly bactericidal and destroyed *Staph. aureus* and *Ps. aeruginosa* in 1 mm. i.e. a >5 Log<sub>10</sub> reduction (99.999%) in the presence of light and moderate soiling (1% serum). However, the disinfectant failed to achieve a 5 Log<sub>10</sub> reduction in *Myco. terrae* (test strain for tuberculocidal activity) and a 6 Log<sub>10</sub> reduction in *Bacillus subtilis* spores with contact times of 60 mm and 6 hrs respectively. Sterichelle S-2 000 at a use dilution of 1 part in 63 parts of standard hard water could be described as a disinfectant as it is effective against *Staph. aureus* and *Ps. aeruginosa* but not as a high level disinfectant or 'sterilant'. Rapid

tuberculocidal and sporicidal activity are necessary for heat sensitive critical and semi critical instruments such as endoscopes. We recommend further testing with spores and mycobacteria at a higher use concentration. Other essential selection criteria are instrument/processor compatibility, user and environmental safety and cost. Data is available on the sporicidal and tuberculocidal activity of a wide range of instrument disinfectants using the same test methodology if this is required.

**CR Bradiey**  
**Senior MLSO**

**JR Babb**  
**Laboratory Manager**

**Dr AP Fraise**  
**Director**