Sporocidal Activity of Suprox(Neutral Anolyte) Liquids

Please note these are qualitative not quantitative test. A local fully accredited laboratory was used to buy the plates and media from. All procedures must be carried out using sterile apparatus, with appropriate aseptic technique, and with due regard to safe disposal of all materials.

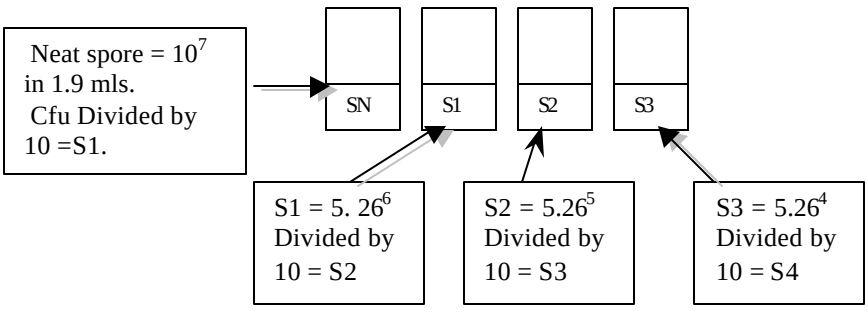
Preparation of spores and mix cultures (MC)

Basillus Subtilus Var Niger NCTC 100073 in Nutrient Broth was the bacterium used for the spore test. It was purchased from Tepnel BioAnalysis at a log strength of 7/8 (thus allowing use of their traceability), spores were kept at –5°c until used for the test. The spores were taken from the nutrient broth and 1ml was placed into a sterile universal containing 20ml of nutrient broth this was then incubated for 48h. Prior to use heat the spore solution to70°c for 30mins to inactivate growing cells. Agar plates were also purchased from Tepnel again using all their traceability of products.

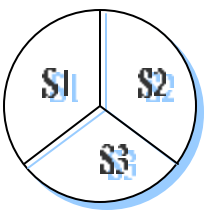
Preparation of mixed cultures was completed in a similar manner as the spores, as with the spores the organisms (*Staphylococcus aureus NCTC, Eschericha Coliforms NCTC,*
Salmonella Tennessee NCTC, in nutrient broth to a log count of log 8-9, were purchased from Tepnel BioAnalysis and stored at –5°c. Four hours prior to the experiment aseptically take 0.1ml of each organism in solution and add to 20mls of nutrient broth in a sterile universal, incubate at 37°c till needed (between 4-5h). It is important to note that the bacterium are not inactivated in this methodology.

Outline of procedures used for spore and mix culture (MC) tests

Add 1.9ml of the Anolyte to be tested to 0.1ml of the mixed culture / neutralized spore solution. Leave for the required time 2 or 15 minuets and neutralize 1ml in 9ml of neutralizing solution, this being double strength nutrient broth with 1% sodium thiosulphate, 0.75% Lecithin Tween 80 and made using distilled water. Having given the sample 5mins to neutralize the anti-microbial effects of the lotion, use serial dilutions (1ml in 9mls) to test the working area of the anolyte being tested. Make three, 1 in 10 dilutions of the neutralized solution and streak a sterile loop full of each dilution on to a segmented plate (as below) to check for growth or no growth in that dilution range. The agar plates are made of tryptone soya agar (TSA) and are incubated for 24 & 48hours, at 37°c



Label plates as below and using sterile spreaders, streak each dilution on to agar plate.



Enumeration of spore / mix culture solution.

The enumeration of spores (see how strong the spore solution actually is) prepare the culture solutions (spore or mixed cultures) in the manner as usual then instead of mixing with anoyte mix 0.1ml of spores / mix culture with 2.9mls of nutrient broth this is then left for 5mins, after 5mins has elapsed complete several serial dilutions (6 in total). Plate out 1ml per dilution on to TSAgar and spread. Invert the plates and Incubate for 48h at 37^{o}c**,** below is a diagram of the six dilutions completed;

Enumeration post incubation, if there are 14 colonies on the S6 dilution plate this refers to the neat strength of the bacterial / spore solution having 14 colonies, add a 0 for each dilution, therefore giving a S1 strength of 14 x 10^6, S2 strength of 14 x 10^5 and so forth.

Suprox liquid

Suprox liquids produced on a suprox generator were air tight and stored in a dark place at room temperature. The minimal saple size is 1 liter, having physiological properteies in the rang of;

pH 7-8 **ORP** >750mv **C.ac** ~500ppm/l

Sporocidal and Mix Culture Results

Period of time before test	Log reductions after period of time for 'suprox' liquids			
	Spore culture contact time		Mixed culture contact time	
	2min	15min	2min	15min
Week 1	Log 6	Log 6	Log 6	Log 6
Week 2	Log 6	Log 6	Log 6	Log 6
Week 3	Growth on all dilutions	Log 6	Log 6	Log 6
Week 4	*	Log 6	Log 6	Log 6
Week 5	*	Log 6	Log 6	Log 6
Week 6	*	Log 6	Log 6	Log 6
Week 7	*	Log 5	Log 6	Log 6
Week 8	*	Log 3	Log 6	Log 6
Week 9	*	Log 3	Log 6	Log 6
Week 10	*	Growth on all dilutions	Log 6	Log 6
Month 4	*	*	Log 6	Log 6
Month 5	*	*	Log 6	Log 6
Month 6	*	*	Log 6	Log 6
Month 7	*	*	Log 6	Log 6
Month 8	*	*	Log 3	Log 6
Month 9	*	*	Growth on all dilutions	Log 5

* = not tested further due to failing (having growth)..

All tests are on going, on dilutions still having an effect on S3 (log 3/4 reductions).

Longitudinal Sporicidal Results for Emerald Configuration

	Sporicidal reduction in **15 minuet** contact time							
Sample	Week 1	Week 2	Week 3	Week 4	Week5	Week6	Week 7	Week8
Suprox Liquid 18v and 25v	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{5}	Log^{3}

Longitudinal Mixed Culture Results for Emerald Configuration

	Biocidal reduction in **2 minuet** contact time					
Sample	Month 4	Month 5	Month 6	Month 7	Month 7.25 Week 29	Month 8
Suprox Liquid 18v	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{5}	Log^{3}

	Biocidal reduction in **15 minuet** contact time				
Sample	Month 4	Month 5	Month 6	Month 7	Month 8
Suprox Liquid 18v	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}

The Biocidal (Mixed Culture Results) were only completed after a 3 month period had elapsed due to the test not being used until then. All the above tests are ongoing and further data will be made available at a later date.

Below are the Results above, re-hashed and re-writen

	Sporicidal reduction in **15 minuet** contact time							
Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Suprox 18v	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{5}	Log^{3}
Suprox 25v	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{5}	Log^{3}
Supros 36v	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{5}	Log^{3}

	Biocidal (Mixed Culture) reduction in **2 minuet** contact time								
Sample	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 7.25	Month 8
Suprox 18v				Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{5}	Log^{3}
Suprox 25v	Log^{6}	Log^{6}	Log^{6}	Log^{6}					
Suprox 36v	Log^{6}	Log^{6}							

	Biocidal (Mixed Culture) reduction in **15 minuet** contact time								
Sample	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 7.25	Month 8
Suprox 18v				Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}	Log^{6}
Suprox 25v	Log^{6}	Log^{6}	Log^{6}	Log^{6}					
Suprox 36v	Log^{6}	Log^{6}							