

Active Clean B

Efficacy Testing

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Aim

The purpose of this trial was to determine the efficacy of Active Clean B used for sanitisation of surfaces.

Background

Previous work has shown the efficacy of sanitising products differs depending on the surfaces to which they are applied. To represent each of these common surfaces, small discs were created from untreated undressed timber, plastic (as used in some bins), rubber (from tyres) and mild steel.

This report presents the evaluation of Active Clean B tested both in-vitro and in spray and dip applications on all 4 surfaces after each surface had been *spiked* with Psa V. This trial includes a number of different testing approaches. Initial tests were undertaken in solution (0.85 % saline) to determine appropriate concentrations for efficacy. The sensitivity of the product to acidity (pH) was then assessed and we finally attempted to assess whether the efficacy of the disinfectant was affected by the presence of organic matter.

Table 1 below shows the dilution of product and the main active ingredient

Dilution of product	Concentration of Active Essential Oil –Blend B at each dilution
1 %	0.025%
2 %	0.05%
3 %	0.075 %
4 %	0.1 %
5 %	0.125%

Table 1: Dilutions and concentration of active ingredient.

Methodology

1. Minimum Inhibitory Concentration (MIC) in 0.85 % normal saline

A Psa-V suspension was made in 0.85% normal saline and quantified in cfu/mL. This assay measures the activity of the chemical agent against Psa-V without the interference of broth ingredients. The tubes were incubated for 48 hours at 25 ±2°C post inoculation after which the tubes were sub-cultured onto Kings B media, incubated for a further 48 hours and the growth of Psa V was characterised. Based on the results obtained in the dilution test, a working concentration of each product was determined for use in the efficacy trial on surfaces.

2. pH sensitivity

The product was tested at progressively higher pH levels by slowly adding phosphate buffer until the desired levels were reached. The tubes were then incubated for 48 hours at 25 ±2°C. At the end of incubation, the tubes were sub-cultured onto Kings B plates and incubated for 48 hours and growth of Psa V was characterised. The aim of the test was to show the correlation of pH sensitivity to products' efficacy.

3. Organic matter sensitivity

In order to test the sensitivity of each product to a quantifiable level of organic matter 0.1% tannic acid was used to create the working concentrations of each product (rather than water) in solution with concentrations of 1, 5, 10 and 20ppm organic matter. The mixture was then tested as per the routine procedure against Psa V: tubes were incubated for 48 hours at 25 ±2°C and then sub-cultured onto Kings B plates and incubated for 48 hours and growth of Psa V characterised.

4. Spray and dip applications

The product was made up to its working concentration prior to spray and dip applications. For each product and material, the prepared spiked discs (1 x 10⁷cfu/mL) were either sprayed or soaked for 10secs, 30secs, 1 minute and 2 minutes in the test products. Following this test the discs were carefully swabbed with a sterile pre-moistened swab. The swab was then immersed into 1 mL 0.85 % saline and plated onto both 1 x media H and 1 x Kings B media. The procedure was repeated for all spray and dip treatments and for each material type. These plates were incubated at 25°C ± 2°C for 2 days before checking for growth of Psa V. A set of discs of each material was spiked without any treatment and swabbed and plated as a positive control.

Results

1. Minimum Inhibitory Concentration (MIC) in 0.85 % normal saline

The minimum inhibitory concentration of each product tested from 1 % to 5% dilution. A working concentration for the product derived from the MIC was used for subsequent testing as per Table 2 below.

Product under test	Psa V solution used in cfu/mL	1%	2%	3%	4%	5%
Active Clean –Blend B	1.3 x 10 ⁷	NG	NG	NG	NG	NG

Table 2: MIC in 0.85 % saline suspension

Key: **G – Growth** **NG- No growth**

1. Effect of pH on efficacy of products

We attempted to adjust the pH of the product solution to achieve 3 different pH levels from the range 4, 5, 6, 7, 8 depending on the pH of the product diluted in Psa-V suspension. The pH of the Psa-V solution is neutral. A fourth undisturbed treatment was left as a control. Measured pH levels are recorded since small volumes and different buffering meant that final pH levels varied.

*Actual pH refers to the unadjusted solution pH

**Adjusted pH is what was achieved by adding small amounts of HCl or phosphate buffer (to increase pH)

Active clean B	Actual pH	Adjusted pH	Growth
1	6.6	N/A	NG
2	6.6	5.1	NG
3	6.6	3.6	NG
4	6.6	7.3	G

Table 3: Natural pH is almost neutral. No pH sensitivity.

2. Effect of organic matter on efficacy of products

This trial is an attempt to indicate any intrinsic sensitivity of the product to the presence of organic matter using a simple, but repeatable, test. Each product was tested at its working concentration determined from the earlier MIC tests. We expected that any product which reacted in a purely chemical way with organic matter might simply react with the entire active ingredient leaving none to react with Psa-V. The results obtained for the product tested are as per Table 4 below.

Product	Product concentration	Concentration of organic matter			
		20 ppm	10 ppm	5 ppm	1 ppm
Active clean B	1 % pH adjusted to 4.5	NG	NG	NG	NG

Table 4: Effect of organic matter on efficacy of product.

Key: **G – Growth** **NG- No growth**

3. Spraying and Dipping Efficacy

A Table of results is presented for Active clean B at the working concentration of 2 % pH adjusted 4.5 showing efficacy on each surface for each application method and for each media type and exposure time.

Active clean B	2 % concentration	Media H				Kings B			
Surface	Application method	10secs	30secs	1 min	2mins	10secs	30secs	1min	2 mins
wood	Spray	NG	NG	NG	NG	NG	NG	NG	NG
	dip	NG	NG	NG	NG	NG	NG	NG	NG
Plastic	Spray	NG	NG	NG	NG	NG	NG	NG	NG
	dip	NG	NG	NG	NG	NG	NG	NG	NG
Rubber	Spray	G	NG	NG	NG	G	NG	NG	NG
	dip	G	NG	NG	NG	G	NG	NG	NG
Metal	Spray	G	NG	NG	NG	G	NG	NG	NG
	dip	G	G	G	NG	G	G	G	NG

Table 5: Active Clean B surface disinfectant study

Key: **G – Growth** **NG- No growth**

Summary

Active clean B showed efficacy at 10 seconds exposure for wood and plastic for both dip and spray applications. It showed efficacy at 30 seconds exposure to metal and rubber for both applications except for metal where absolute kill was observed at 2 minutes for the dip application.

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