AUSSAN L-44 EFFICACY TESTING

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Aussan L- 44 Efficacy Testing

Product	Aussan L-44
Batch No	20122012
Active ingredients (Label)	Bitter orange extract (20-30 %), Lactic acid(5-10%) and water & non-hazardous (Rem)
Mode of action	Biological
Application rate	0.3 % dilution

Aim:

The purpose of this trial was to determine the efficacy of Aussan L-44 used for sanitisation of surface.

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 Aussan-L44 (0.3 %) tested both in-vitro and in spray and dip applications on all 4 surfaces after each surface had been *spiked* with Psa V.

Methodology

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

A suspension of Psa-V was made in 0.85% normal saline and quantified in cfu/mL. The dilution test was repeated using a similar or narrower dilution range based on the outcome of the broth dilution test carried out previously. This assay measures the activity of the chemical agent against Psa-V without the interference of the 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 combined dilution tests, a working concentration of each product was determined for use in the efficacy trial on surfaces.

2. 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 10^8 cfu/mL) were either sprayed or soaked for 10s, 30s, 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.

3 Scoring

After incubations, all plates were read and each plate was scored using a zero to 4 scales where 4 correspond to abundant growth of Psa and zero is no growth as per Table 1 below.

Growth score	Psa growth
G1	< or equal to 25% Psa growth
G2	50 % Psa growth
G3	75% Psa growth
G4	100 % Psa growth
NG	No growth

Table 1: Growth Score

Results

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

The working concentration was found to be **0.3 %** and was used for subsequent testing.

Product under test	Quantification in cfu/mL	Psa in 0.85 % saline					
		0.1%	0.15%	0.2%	0.25%	0.3 %	0.35%
Aussan L-44	1 x10^8 cfu/mL	G	G	G	G	NG	NG

Table 2: MIC in 0.85 % saline suspension

Key: ***G – Growth*** ***NG- No growth***

2. Spraying and Dipping Efficacy

Aussan L-44 used at 0.3 % working concentration showed efficacy at 30 seconds exposure and longer in spray and dip application for all surfaces except for metal. Refer to Table 3.

Aussan L-44	0.3 % concentration	Media -H (Psa selective media)				Kings B media			
Surface	Application method	10sec	30sec	1min	2min	10sec	30sec	1 min	2 min
Wood	Spray	NG	NG	NG	NG	NG	NG	NG	NG
	Dip	G	NG	NG	NG	G	NG	NG	NG
Plastic	Spray	NG	NG	NG	NG	NG	NG	NG	NG
	Dip	G	NG	NG	NG	G	NG	NG	NG
Tyre	Spray	NG	NG	NG	NG	NG	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	NG	NG	G	G	NG	NG

Table 3: Aussan L 44 efficacy results at 0.3 %

*Key: **G – Growth** **NG- No growth***

Scoring of plates 4: 100 % growth 3: 75 % growth 2: 50 % growth, 1 <25 % growth

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