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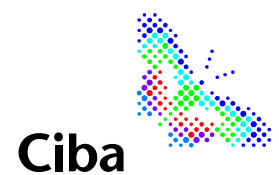
Determination of the antibacterial activity of an I&I disinfectant,
Microbicide, containing tinosan[®]sdc

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Determination of the antibacterial activity of Microbecide, an I&I disinfectant containing tinosan® sdc (MIC)

Introduction

The **Minimal Inhibitory Concentration (MIC)** of an I&I disinfectant, **Microbecide**, containing tinosan® sdc has been determined according to the modified Ciba Standard CG 128e against a broad spectrum of microorganisms. (**MIC** denotes the minimum concentration of the test product to inhibit the growth of the organisms). The MIC's are given in a table.

In a second study the bactericidal activity of the I&I Disinfectant, **Microbecide**, has been determined according to the European Standard EN 1040 (Chemical disinfectants and antiseptics / Basic bactericidal activity / Test method and requirements phase I)

Conclusion

The I&I disinfectant "**Microbecide 30ppm**" containing tinosan® sdc show a **Minimal Inhibitory Concentration (MIC)** against *Staphylococcus aureus* ATCC 6538 and the yeast *Candida albicans* ATCC 10231 of 10% and 1% against *Corynebacterium minutissimum* ATCC 23348 and *Escherichia coli* ATCC 10536.

Therefore a 10% dilution of the I&I disinfectant is still active against a broad spectrum of microorganisms (Gram-positive, Gram-negative and Yeasts).

Bactericidal activity

At a concentration of 90% and 10% the I&I Disinfectant "**Microbecide 30 ppm**" fulfills European Standard EN1040 at a test temperature of 22°C the required log reduction (> 5 log ie >99.999% reduction) after 5 minutes contact time against both strains tested (*Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442).

1) Determination of the Minimum Inhibitory Concentration (MIC) according to the modified Ciba Standard CG 128e

Samples: I&I disinfectant
Microbicide (30ppm) BIN: 08807P containing 30 ppm TINOSAN® SDC

Strains: Staphylococcus aureus ATCC 6538
Corynebacterium minutissimum ATCC 23348
Escherichia coli ATCC 10536
Candida albicans ATCC 10231

Nutrient agar: Muller Hinton agar

Principle

The substance to be tested is incorporated by serial dilutions in the nutrient medium. The microorganisms are then inoculated at different points on the agar and incubated. After incubation, the concentration beyond which no microbial growth takes place is determined.

Serial dilutions are made in sterile water.

The final concentrations in the agar plates are the following:

10% / 1% / 0.1% / 0

All prepared agar plates are stored overnight in the laboratory (at about 22°C) for drying.

A liquid 18 - 24-hour bacterial culture in 5 ml Tryptic soy broth (has to contain approximately 10^8 - 10^9 cfu/ml) is diluted 1:10 - 1:100, (depending on strain) with sterile sodium chloride solution 0.85%, pH 7.2 (final concentration: $\sim 10^7$ cfu/ml)

Inoculation per plate with a micropipette: 10µl / strain

Incubation conditions: 24 - 48 hours at 37°C

Results

Microorganisms	Tested concentrations			MIC (% solution)
	0.1%	1%	10%	
I&I disinfectant Microbecide (30ppm) BIN: 08807P containing tinosan [®] sdc	0.1%	1%	10%	
Staphylococcus aureus ATCC 6538	+	+	-	10
Corynebacterium minutissimum ATCC 23348	+	-	-	1
Escherichia coli ATCC 10536	+	-	-	1
Candida albicans ATCC 10231	+	+	-	10

+ = strong growth (no activity)

- = no growth (activity)

Water and agar controls show strong growth with all micro-organisms tested

2) Determination of the bactericidal activity according to the suspension test method EN 1040 (dilution neutralisation)

Sample: I&I disinfectant
Microbecide (30ppm) BIN: 08807P containing 30 ppm tinosan[®] sdc

Test bacteria: Staphylococcus aureus ATCC 6538
Pseudomonas aeruginosa ATCC 15442

Concentration: 90% and 10% (final test concentration)

Contact time: 5 minutes at 22°C

Nutrient medium: Case agar (CA)

Inactivation medium: Trypticase Soya Broth + 10% Tween 80 + 3% lecithin + 0.1% L-histidin + 0.5% Nathiosulfate (TLHNa)

Incubation of plates: 24 - 48 hrs at 37°C

Results (log reduction)

Log bacteria reduction according to EN 1040					
Staphylococcus aureus ATCC 6538 / OD: 0.423 / 3.3×10^8 / ml					
5 minutes contact time					
Test formulations	Cfu/ml	In log	Log red	% red	pH 22°C
Test concentration: 90% I&I disinfectant Microbecide (30ppm) BIN: 08807P containing 30 ppm tinosan [®] sdc	< 100	< 2	> 5	> 99.999	1.8
Test concentration: 10% I&I disinfectant Microbecide (30ppm) BIN: 08807P containing 30 ppm tinosan [®] sdc	< 100	< 2	> 5	> 99.999	2.35
H ₂ O Control (deion. water)	2.6×10^7	7.4	---	---	7.6
Pseudomonas aeruginosa ATCC 15442 / OD: 0.130 / 3.8×10^8 / ml					
5 minutes contact time					
Test formulations	Cfu/ml	In log	Log red	% red	pH 22°C
Test concentration: 90% I&I disinfectant Microbecide (30ppm) BIN: 08807P containing 30 ppm tinosan [®] sdc	< 100	< 2	> 5	> 99.999	1.9
Test concentration: 10% I&I disinfectant Microbecide (30ppm) BIN: 08807P containing 30 ppm tinosan [®] sdc	< 100	< 2	> 5	> 99.999	2.4
H ₂ O Control (deion. water)	2.3×10^7	7.35	---	---	7.6