Identification of The Testing Laboratory: Istanbul University, Faculty of Science, Department of Biology, Section of Fundamental and Industrial Microbiology Laboratory

Identification of The Sample:

1) name of the product: Antimic®

2) batch # 20150706 - expire date July 2017

3) manufacturer: Nanotego A.Ş

4) date of delivery; 24.05.2016

5) storage conditions: At room temperature

6) product diluent recommended by the manufacturer for use; **20-50 ppm**

7) active substance(s) and their concentration(s) (optional): Trimethoxysylil quaternary ammonium compound

8) appearance of the product; yellow, liquid

THE TEST REPORT ON THE EFFICACY OF ANTIMIC® AGAINST ENTEROCOCCUS FAECIUM VRE STANDARD STRAIN

In the current test, the efficacy of Antimic compound was tested against VRE (ATCC 51299).

Various concentrations of Antimic were prepared in sterile deminerilase water:

1- Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Macrodilution method was followed for the determinations the minimum inhibitory concentrations of biocides for *Enterococcus faecium* VRE (ATCC 51299) standard strain.

Different concentrations of biocides were prepared in test tubes and each tube was then inoculated with *E. faecium* inocula (final concentration 3-7 x 10^5 cfu*ml^{-1}). The tubes were incubated at 37 °C for 24-48 hour and then examined for visual turbidity. The lowest concentration of the biocide, at which growth was inhibited (indicated by lack of turbidity), was taken as MIC. Samples of 100 µl were drawn from each tube without turbidity and were subcultured on Mueller Hinton Agar (MHA) plates to determine bactericidal concentration.

(*cfu: colony forming unit)

% 0.0005 (5 ppm) and **% 0.005 (50 ppm)** concentrations of Antimic as were taken **MIC** and **MBC** values, respectively.

Therefore, the bactericidal activity of 50, 25, 20 ppm dosages of the compound were assessed at different contact times (1 and 5 minute), in both clean and dirty conditions by EN 1276 standard method:

BS EN 1276:2009

Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas. Test method and requirements (phase 2, step 1)- Dilution-Neutralization Test Method

Identification of Test Conditions

1- Product Test Concentration: 50, 20, 25 ppm
2- Contact Times: 1 and 5 minute
3- Test Temperature: 20˚C
4- **Interfering Substance:**
Clean Conditions: 0.03% w/v albumin (final concentration)
Dirty Conditions: 0.3% w/v albumin (final concentration)

5- Inhibition Method
Dilution/neutralization
6- Neutralizer
Polysorbate 80 3% (v/v) and lecithin 0·3% (w/v)

In the current test method, the bactericidal activity of 50, 25, 20 ppm biocide concentrations was assessed by adding bacterial suspension into sterile hard water containing different concentrations of biocide.

An 8 ml sample of disinfectant diluted in water of standard hardness (300 mg kg^{-1} CaCO3) at concentrations of 0.0063% (v/v), 0.0032% (v/v) and 0.0025% (v/v) to give final test concentrations of 0.005%, 0.0025% and 0.002% was added to 1 ml bovine albumen serum at concentrations of 0.3% (w/v) and 0.03% (w/v) (to represent dirty and clean conditions, respectively). 1 ml of the bacterial suspension (1.5–5x10^8 cfu ml^{-1}, had been added to this mixture. After a contact time of 1 and 5 minute, 1 ml of the test mixture was pipetted into 8 ml neutralizer (comprising polysorbate 80 3% (v/v) and lecithin 0·3% (w/v) and 1 ml deionized water. After 5 minute neutralization time, duplicate 1 ml volumes were pour plated with tryptone soya agar (TSA) and incubated at 37 °C for 48 h prior to counting.

Three validation (control) procedures were undertaken in parallel for each disinfection test on all occasions as follows:

Validation A. The test was undertaken with the addition of 8 ml water of standard hardness in place of the disinfectant to ensure that there was no biocidal action of the other experimental parameters.
***Validation B*.** Neutralizer (8 ml) and 1 ml water were added to the bacterial suspension and then plated out to ensure that the neutralizer had no toxic effect.
***Validation C*.** Bacterial suspension (1 ml) was added to neutralized disinfectant to ensure that the disinfectant had been neutralized.

For each test, positive controls of treatment (with bacteria and with disinfectant) and negative controls of treatment (with bacteria and without disinfectant) were included. Each assay was repeated in triplicate.

Calculation and Expression of the Results

Calculation of the viable count (cfu/ml)

The count was performed using the number of colonies counted on plates.
Only the plates showing between 15-300 colonies were used to perform the result calculation. A deviation of 10% is accepted, so the limits are 14-330.
In the assay, where the number of cfu on each plate counted is < 14, the number of cfu/ml should be recorded as < $1.4x10^2$.
Where the number of cfu on each plate counted is >330, the number of cfu/ml should be recorded as < $3.3x10^3$.

Test suspension

The calculation of the bacterial count fort he suspension test (N) is performed applying the following Formula:

$$N(cfu/ml) = \frac{c}{(n_1 + 0.1n_2)d}$$

Where:
c: sum of the colonies counted on plates
n_1: number of the counted plates in the lower dilution
n_2: number of the counted plates in the higher dilution
d: dilution factor corresponding to the lower dilution

Calculation of vitality reduction

The reduction in viability was calculated by subtracting the log of the viable count after disinfection (Na) from the log of the initial count in the test chamber ($Nx10^{-1}$).

$Log\ R = log\ N_0 - log\ N_a$

where:
R: Reduction of vitality
N_0: N/10
N_a: bacterial count for the test mixture at the end of the contact time

Results

The test substance is considered bactericidal when the bacterial count for each bacterial strain is reduced by **at least 5 log following 5 minutes contact at 20˚C**

The test substance is considered effective against the test organisms when the bacterial count for each bacterial strain by **at least 5 log following the chosen contact at 20˚C**

BACTERICIDAL ACTIVITY OF ANTIMIC®
EN 1276 PHASE 2 STEP 1

Antimic Dosages	Log_{10} Initial Count (mean)	Contact Time	Log_{10} Reduction Achieved	
			Clean Conditions (0.3% w/v albumin)	Dirty Conditions (3% w/v albumin)
50 ppm	7.58	1 min	> 6.44	> 6.44
		5 min	> 6.44	> 6.44
25 ppm	7.58	1 min	5.09	5.05
		5 min	> 6.44	6.09
20 ppm	7.58	1 min	4.69	4.26
		5 min	5.7	5.18
10 ppm	7.58	1 min	5.02	4.02
		5 min	5.06	5.05

CONCLUSİON

On the basis of the results obtained, in compliance with the assay validity criteria, Antimic ® results **BACTERICIDAL** with the concentration of **% 0.005 (50 ppm) and % 0.0025 25 ppm after 1 minute and 5 minutes of contact times, using** 3% w/v and 0.3% w/v bovine albumin, in compliance with EN 1276:2009.

Also, Antimic ® has provided > 5 log redution criteria, with the concentration of **% 0.002 (20 ppm) and % 0.001 10 ppm after 5 minutes of contact times, using** 3% w/v and 0.3% w/v bovine albumin, in compliance with EN 1276:2009.

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