

Analysis report. Microbiology Department.**Sample information**

Product: LIQUIDNATURE and
NEBUNATURE (DOMCA SAU)

Petitioner

Name: Andrea Ricci
Company: Sales Director DOMCA

A) Requested analysis: Evaluation of antimicrobial activity

B) Methodology

- Microorganisms: *E. coli* DMC-02, *L. monocytogenes* CECT 4032, *Salmonella enterica* CECT 4395, *Staphylococcus aureus* DMC-015, *Candida albicans* DMC-07 and *Penicillium expansum* DMC-05.
- Cultured medium: MacConkey agar, Rose Bengale Agar, Saboureaud agar and Compass Listeria (Bioser Diagnostics)
- Determination of the Minimum Bactericidal Concentration (MBC)** was performed by the broth microdilution method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) collected in the standard Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically [42], in order to establish the lowest concentration of each antimicrobial agent that reduces the viability of initial inoculum by 99.9%. 1:2 decreasing dilution were prepared from an initial solution of each product at 10,000 µg/ml so that the following concentrations were obtained: 5,000; 2,500; 1,250; 625; 312.5; 156.25; 78.125; 39.06; 19.53; and 9.76 µg/ml. Each dilution was inoculated with a microbial suspension so that the final concentration in each well was 10⁵ CFU/ml, and incubated overnight at room temperature. As a positive control, a mix of ketoconazole, ampicillin and streptomycin (100,000 µg/ml) was used. As a negative control, liquid media without an antimicrobial agent was inoculated with microorganisms. Microbial growth was tested by culturing in agar plates, and the lowest concentration of products in which no growth was observed was established as the MBC.

*CLSI, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 11th ed. 2018.

C) Results

Table 1. Minimum Biocidal Concentration (MBC). Result expressed in mg/L

	LIQUIDNATURE	NEBUNATURE
<i>Escherichia coli</i>	156.25	78.12
<i>Salmonella enterica</i>	625	625
<i>Listeria monocytogenes</i>	312.5	312.5
<i>Staphylococcus aureus</i>	312.5	625
<i>Candida albicans</i>	156.25	156.25
<i>Penicillium expansum</i>	625	1250

Date: 13th of January 2023

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