Micro-B
LABORATORIO ANALISI CHIMICHE E MICROBIOLOGICHE

MICRO-B s.r.l.
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Reg. Imp: MN02140460201 R.E.A. MN227554Cap. Soc. i.v: €30.000,00

Certificate n. 2001216-003 Asola, 14/04/2020

Client: BONASYSTEMS ITALIA SRL
Via Borgo S. Chiara, 29 – Torre di Mosto (VE)

Sample number: 2001216-003
Test run: 08/04/2020
Sample recording: TEST B ZERO
Specimen dimension: 50x50 mm
Sterile film used: polyethylene 40 x 40 mm, sp. 0.11 mm
Strain:
Escherichia coli ATCC 8739 (8.4 x 10^5 ufc/ml)
Staphylococcus aureus ATCC 6538 (5.1 x 10^5 ufc/ml)
Samples: by customer
Sampling method: customer's care

Sample arrival data: 13/03/2020
Test report: 14/04/2020

Verification of the effectiveness of the "BACTERI ZERO" sanitizing cleaner after 10 applications on porcelain stoneware and subsequent measurement of antibacterial activity using the ISO 22196: 2011 method
Measurement of antibacterial activity on plastics and other non-porous surfaces

1. TEST SCOPE

Verification of the procedure for using BZERO, the action of which is achieved through its cumulative and additive capacity to modify the chemical / physical characteristics of the surfaces.

This method is applicable for evaluating the antibacterial activity of antibacterial-treated plastics, and other non-porous, surfaces of products.

Test material is inoculated with a known amount of bacteria suspension inoculum; the amount of bacteria is then measured after 24 hours time contact. The comparison between the two quantities provides a percentage index R of effectiveness of the antimicrobial material.

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2. STAGES OF THE ASSAY

Stages of the treatment of porcelain stoneware tiles with the universal multipurpose cleaner "B ZERO" are:

1. Wet the microfibre cloth with water (impregnation and wringing).
2. Impregnation of the Micron Quick Vileda microfibre cloth n ° 134859 0373 with the universal multipurpose cleaner "b zero".
3. Wringing of excess product.
4. Cleaning of porcelain tiles with light and regular passage.
5. Natural air drying of the humidity released on the surface.

This procedure was repeated 10 times.

Test method ISO 22196: 2011 involves the following steps:

1. Preparation of bacterial inoculum.
2. Inoculum of bacteria on treated and untreated specimen. Cover with sterile film.
3. Incubation at 35°C for 24 hours.
4. Washing of specimen with Neutralising sample diluent; pour plate technique for bacterial count.
5. Evaluation of test results and calculation of the antibacterial activity of the treated material.

3. MICROORGANISM FOR INOCULATION

The microorganism used for contaminations is:

✶ *Escherichia coli* ATCC 25922

Eschericia coli, also known as E. coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Pathogenic strains of E. coli are responsible for urinary tract infections, intestinal disorders such as gastroenteritis and neonatal meningitis. It is the main indicator of faecal contamination.

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✶ *Staphylococcus aureus* ATCC 6538. Staphylococcus aureus is a Gram-positive, round-shaped bacterium. It is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. Strains of S. aureus are responsible for skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning.

Standard lyophilized cultures of microorganism were used.
The bacterial inoculum size is between 2.5 x 10^5 and 1.0 x 10^6 cfu / ml.

4. CULTURE MEDIA

To perform the experimental test were used culture media, such as:

- Phosphate-buffered physiological saline (PBS) solution for the preparation of microbial suspensions of standard strains used and serial dilutions;
- Plate Count Agar (PCA) for the method of sowing in inclusion in the Petri dish.
- Specific neutralizing thinner for the final step of testing.

5. TEST PROCEDURE

In order to verify the procedure of use of BZERO, whose action is achieved through its cumulative and additional ability to modify the chemical / physical characteristics of the surfaces, the product is applied for 10 consecutive times following the instructions on the back of the product packaging . Once the specimens have been treated, proceed to ISO 22196: 2011.
Antibacterial activity is evaluated by measured the viability of bacteria after contact with a surface treated with antibacterial agents, for 24 hours at 35°C.
The effectiveness of antibacterial agents is measured by comparing the degree of survival of the bacteria put in contact with treated and untreated materials.

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The strains of *Escherichia coli and Staphylococcus aureus* is inoculated into a nutrient broth (PBS). An aliquot of this culture is put on 3 sample of surface treated with antibacterial agents; an other aliquot is put on 3 sample with untreated surface. All samples are then divided in 50x50mm portions and inoculated with bacterial inoculum; the surface is covered with 40x40mm sterile film.
The samples are incubated at 35°C for 24 hours with 90% of humidity.
After incubation, 10 ml of Neutralising sample diluent are added to all the samples (treated and untreated); an aliquot of the Neutralising sample diluent is then plated onto growth medium PCA, for bacterial count.

6. RESULTS

From the microbial count results obtained, anti-bacterial activity R is calculated with the equation given in ISO 22196: 2011.
The analytical results are intended to refer exclusively to the analysed samples received at the laboratory. This document cannot be reproduced even in partial form unless written approval by the Laboratory.

TEST RESULTS WITH ESCHERICHIA COLI

Initial bacterial count (CFU/cm^2) **Uo**	Bacterial count after 24h on untreated samples (CFU/cm^2) **Ut**	Bacterial count after 24h on treated samples (CFU/cm^2) **At**	**Antibacterial activity R= Ut – At**	**Reduction (%)**
5.3×10^4 Log = 4.72	4.8×10^5 Log = 5.68	1.1×10^5 Log = 5.04	**0.64**	**77.08**

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TEST RESULTS WITH STAPHYLOCOCCUS AUREUS

Initial bacterial count (CFU/cm^2) **Uo**	Bacterial count after 24h on untreated samples (CFU/cm^2) **Ut**	Bacterial count after 24h on treated samples (CFU/cm^2) **At**	**Antibacterial activity R= Ut – At**	**Reduction (%)**
9.1×10^4 Log = 4.96	8.6×10^5 Log = 5.93	2.3×10^5 Log = 5.36	**0.57**	**73.26**

7. INTERPRETATION OF RESULTS

R value represented the anti-bacterial activity. The number expressed is the ability to eliminate on a logarithmic basis, in 24 hours, the bacteria that are in contact with the surface treated with antibacterial agent.

The more R factor is high, the more the treated surface has effectiveness to kill bacteria and prevent formation of CFU.

Micro-b s.r.l.
Technical Manager
Dott. Matteo Sarzi Amade'