ACTIVITY VALIDATION PROTOCOL

Bactericide, Yeasticide, Fungicide

of an SDD (STEAM DISINFECTION DEVICE)

EV INTERNATIONAL

ATLANTIS X-Model GV2026
According to EN 13697:2015 + A1:2019 and AFNOR T72-110



Product Name:	ATLANTIS X - Model GV2026 continuous refilling
Client:	E V International Ltd. Kostur 14 A 1618 Sofia, Bulgaria
Date Received:	24/06/2026
Analysis Start Date:	24/06/2026
End Date Analysis:	30/06/2026
Indications for use:	As stated in the report

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1. FOREWORD

The present work was drawn up by the Sana srl laboratory, accredited ACCREDIA at no. 0747 to the UNI EN ISO 17025:2005 standard, and was aimed at verifying the bactericidal, yesasticidal, fungicidal action of the system called ATLANTIS X, a dry steam dispensing system at 185°C.

2. DESCRIPTION OF ACTIVITIES

This validation protocol was drawn up to validate the bactericidal activity of the product described above. The tests performed on the product ATLANTIS X with dry steam dispensing at 185°C were carried out following the reference standards as indicated in the table below:

ACTIVITY	MICRORGANISMS	STANDARD USED	% OF USE	CONDITIONS		TIPOLOGY of ACTION
				Dirty 3g *	Clean 0.3 g*	
BACTERICIDE	Pseudomonas Aeruginosa (ATCC 15442) StaphylococcusAureus (ATCC 6538) Escherichia Coli (ATCC 10536)	EN 13697:2015 + A1:2019 (Phase 2, Step 2)	TAL WHAT	YES	YES	5 seconds
						20 seconds
YEASTICIDE	Candida Albicans (ATCC 10231)			YES	YES	5 seconds
						20 seconds
FUNGICIDE	Aspergillus Brasiliensis (ATCC16404)			YES	YES	5 seconds
						20 seconds
BACTERICIDE	Staphylococcus Aureus (ATCC 6538)	AFNOR T72-110	TAL WHAT	YES**	YES	5 seconds
						20 seconds

**interfering* substance*: bovine serum albumin 3.0 %*

***interfering substance:* bovine serum albumin 3% with skimmed milk 1/20

The method is based on quantitative suspension tests for evaluating the bactericidal activity of products with sanitising activity, chemical disinfectants used in the food, industrial, domestic and institutional sectors. In this case, the method was applied to a dry steam dispensing system, so it was also necessary to study bactericidal activity according to the AFNOR T72-110 method dedicated to SDD (STEAM DISINFECTION DEVICES).

3. TERMS AND DEFINITIONS

- **Bactericide**: Product which, under defined conditions, is capable of killing vegetative bacterial forms.
- **Bactericidal activity**: Ability of the product to give rise to a reduction of at least 10^4 in the number of viable bacterial cells belonging to the reference strains *of Pseudomonas Aeruginosa, Escherichia Coli* and *Staphylococcus Aureus* under standard *surface* experimental conditions.
- **Yeasticidal/fungicidal activity**: Ability of the product to give rise to a reduction of at least 10^3 in the number of viable bacterial cells belonging to the reference strains *of Candida Albicans and Aspergillus Brasiliensis* under standard experimental *surface* conditions.
- **SDD (STEAM DISINFECTION DEVICE):** Disinfection system

4. BACTERICIDE ACTIVITY (Phase 2, Step2) EN 13697:2015+ A1:2019

The aim of the study is to demonstrate and evaluate the efficacy of a product on aerobic and anaerobic microorganisms at different concentrations of use on surfaces.
The procedural steps consist of:

a) Preliminary microbial count on samples to be tested
b) Preparation of micro-organisms for inoculation
c) Inoculation of samples on surfaces
d) Checking the survival of micro-organisms after the test contact time
e) Evaluation of results
f) Neutralising validation

Materials and Reagents

Culture Media

For storage of bacterial strains and determination of viable unit counts.

For bacteria

Tryptone Soy Agar (TSA) Composition: Tryptone, pancreatic digestion of casein 15.0 g; Soy peptones, papain digestion of soybean meal 5.0 g; NaCl 5.0 g; agar 15.0 g in 1000 ml pyrogen-free distilled water. Sterilisation in autoclave. pH control at 7.2 ± 0.2 at 20 ± 1 °C.

Diluent

Sodium chloride triptone solution consisting of:
NaCl 8.5 g; Tryptone, pancreatic digestion of casein 1.0 g in 1000 ml pyrogen-free distilled water. pH control = 7.0 ± 0.2 at 20 °C. Autoclave sterilisation.

Neutraliser

The neutraliser has been validated for the tested product.
Composition: Lecithin 10 g/l; Polysorbate 80 50 g/l; Sodium thiosulphate 10 g/l; L-Histidine 0.25 g/l; Phosphate buffer 0.0025 ml/l1 g/l and distilled water at 1000 ml. pH control at 7.0 ± 0.2 at a temperature of 20 ±1°C. Autoclave sterilisation

Analytical technique:

Inclusion seeding technique in Petri dish.

Assay performance (Na): Surface method

For each bacterial strain separately, a test tube was prepared containing 9 ml of *microbial test suspension* with a concentration of between 1.5 x10^8 and 5.0 x10^8 cfu/ml for the bacteria at the test temperature of 20°C ± 1°C.

A previously sterilised steel surface contaminated with a mixture consisting of the test suspension and the interfering substance (consisting of bovine serum albumin and mutton erythrocytes), was placed in direct contact with the product as per the procedure of the reference standard.

At the end of the one-minute contact time, the neutralising agent was added and the plate was then seeded

Incubation conditions

The plates were incubated at 36 °C ±1 °C for 48 h ÷ 96 h for bacteria. After incubation, the number (Vc) of colony-forming units/plate was then determined for each dilution by calculating the value of the test mixture (Na).

Evaluation and calculation of results

Counting was performed using the number of colonies counted on both plates. Only plates containing 15 to 300 colonies were used for the calculation of the results. The standard deviation of 10% equal to the number of colonies of 14 and 330 is accepted.

In plates where the number of colonies on all counted plates was greater than 330, the number of cfu/ml was recorded as >330.

Bacterial suspension N and N_W

The total count is calculated as follows:

$$N\left(\frac{ufc}{ml}\right) = \frac{c}{(n_1 + 0.1n_2)d}$$

where:

- N = number of cells per ml in test suspension
- c = sum of colonies counted on both plates
- n_1 = number of plates counted of the lowest dilution (10^{-6})
- n_2 = number of plates counted of the highest dilution (10^{-7})
- d = dilution factor

$$N\left(\frac{ufc}{ml}\right) = \frac{c * 10}{n * 10^{-5}}$$

where:

- N = number of cells per ml in the test mixture
- c = sum of colonies counted on both plates
- n = number of plates counted of the lowest dilution (10^{-5})

Calculation of Viability Reduction

For each test micro-organism and test concentration of the product, the reduction of live cells was calculated as follows

$$R = \frac{N_w}{N_a}$$

where

- R = reduction in viability
- N_w = test mixture count *(V_c)*
- N_a = test mixture count at the end of the contact time

The reduction ($R = N_w/N_a$) is expressed as a logarithm for each micro-organism.
The number of cfu/ml in the test mixture N_w is therefore recorded.
For each product concentration and each experimental condition, the decimal logarithmic reduction was calculated and recorded using the equation

$$\log R = (\log N_w - \log N_a)$$

Validation of the non-toxicity of the neutraliser

A 1.0 ml of water was added to 8.0 ml of neutraliser and 1.0 ml of bacterial suspension was added in a range of $3.0x10^4$ to $1.6x10^5$ cfu/ml.
After shaking on Vortex and after the neutralisation time of 5 minutes ± 10 seconds, the mixture was diluted with diluent at serial decimal dilutions of 10^{-1} to 10^{-3} .

1 ml was taken, in duplicate, from the mixture and its dilutions and placed in Petri dishes to perform an inclusion count for the bacteria in TSA respectively.

After incubation at 36 °C ± 1 °C for 48 h ÷ 96 h, the number of colony-forming units/plate for each dilution, expressed in cfu/ml, was then determined, verifying the non-toxicity of the neutralising agent used for the *surface* antimicrobial efficacy test.

5. LIEVITICIDE/FUNGICIDE ACTIVITY (Phase 2, Step2) EN 13697:2015+ A1:2019

The aim of the study is to demonstrate and evaluate the efficacy of a product on aerobic and anaerobic microorganisms at different concentrations of use on surfaces.
The procedural steps consist of:

1) Preliminary microbial count on samples to be tested
2) Preparation of micro-organisms for inoculation
3) Inoculation of samples on surfaces
4) Checking the survival of micro-organisms after the test contact time
5) Evaluation of results
6) Neutralising validation

Materials and Reagents

Culture Media

For storage of bacterial strains and determination of viable unit counts.

For MUSHROOMS

Malt extract agar (MEA) Composition: Malt extract 30.0 g; agar 15.0 g in 1000 ml pyrogen-free distilled water. Sterilisation in autoclave. pH control at 5.6 ± 0.2 at 20 ± 1 °C.

Diluent

Sodium chloride triptone solution consisting of:
NaCl 8.5 g; Tryptone, pancreatic digestion of casein 1.0 g in 1000 ml pyrogen-free distilled water. pH control = 7.0 ± 0.2 at 20 °C. Autoclave sterilisation.

Neutraliser

The neutraliser has been validated for the tested product.
Composition: Lecithin 10 g/l; Polysorbate 80 50 g/l; Sodium thiosulphate 10 g/l; L-Histidine 0.25 g/l; Phosphate buffer 0.0025 ml/l1 g/l and distilled water at 1000 ml. pH control at 7.0 ± 0.2 at a temperature of 20 ±1°C. Autoclave sterilisation

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Inclusion seeding technique in Petri dish.

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For each bacterial strain separately, a test tube was prepared containing 9 ml of *microbial test suspension* with a concentration of between 1.5 x10^8 and 5.0 x10^8 cfu/ml for the bacteria at the test temperature of 20°C ± 1°C.

A previously sterilised steel surface contaminated with a mixture consisting of the test suspension and the interfering substance (consisting of bovine serum albumin and mutton erythrocytes), was placed in direct contact with the product as per the procedure of the reference standard.

At the end of the one-minute contact time, the neutralising agent was added and the plate was then seeded

Incubation conditions

The plates were incubated at 30 °C ±1 °C for 18 h ÷ 24 h for Candida Albicans and 42 h ÷ 48 h for Aspergillus Brasiliensis. After incubation, the number (Vc) of colony-forming units/plate was then determined for each dilution by calculating the value of the test mixture (Na).

Evaluation and calculation of results

Counting was performed using the number of colonies counted on both plates. Only plates containing 15 to 300 colonies were used for the calculation of the results. The standard deviation of 10% equal to the number of colonies of 14 and 330 is accepted.

In plates where the number of colonies on all counted plates was greater than 330, the number of cfu/ml was recorded as >330.

Bacterial suspension N and N_W

The total count is calculated as follows:

$$N\left(\frac{ufc}{ml}\right) = \frac{c}{(n_1 + 0.1n_2)d}$$

where:

- N = number of cells per ml in test suspension

- c = sum of colonies counted on both plates
- n_1 = number of plates counted of the lowest dilution (10^{-6})
- n_2 = number of plates counted of the highest dilution (10^{-7})
- d = dilution factor

$$N\left(\frac{ufc}{ml}\right) = \frac{c * 10}{n * 10^{-5}}$$

where:

- N = number of cells per ml in the test mixture
- c = sum of colonies counted on both plates
- n= number of plates counted of the lowest dilution (10^{-5})

Calculation of Viability Reduction

For each test micro-organism and test concentration of the product, the reduction of live cells was calculated as follows

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- N_a = test mixture count at the end of the contact time

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Validation of the non-toxicity of the neutraliser

A 1.0 ml of water was added to 8.0 ml of neutraliser and 1.0 ml of bacterial suspension was added in a range of 3.0×10^4 to 1.6×10^5 cfu/ml.
After shaking on Vortex and after the neutralisation time of 5 minutes ± 10 seconds, the mixture was diluted with diluent at serial decimal dilutions of 10^{-1} to 10^{-3} .

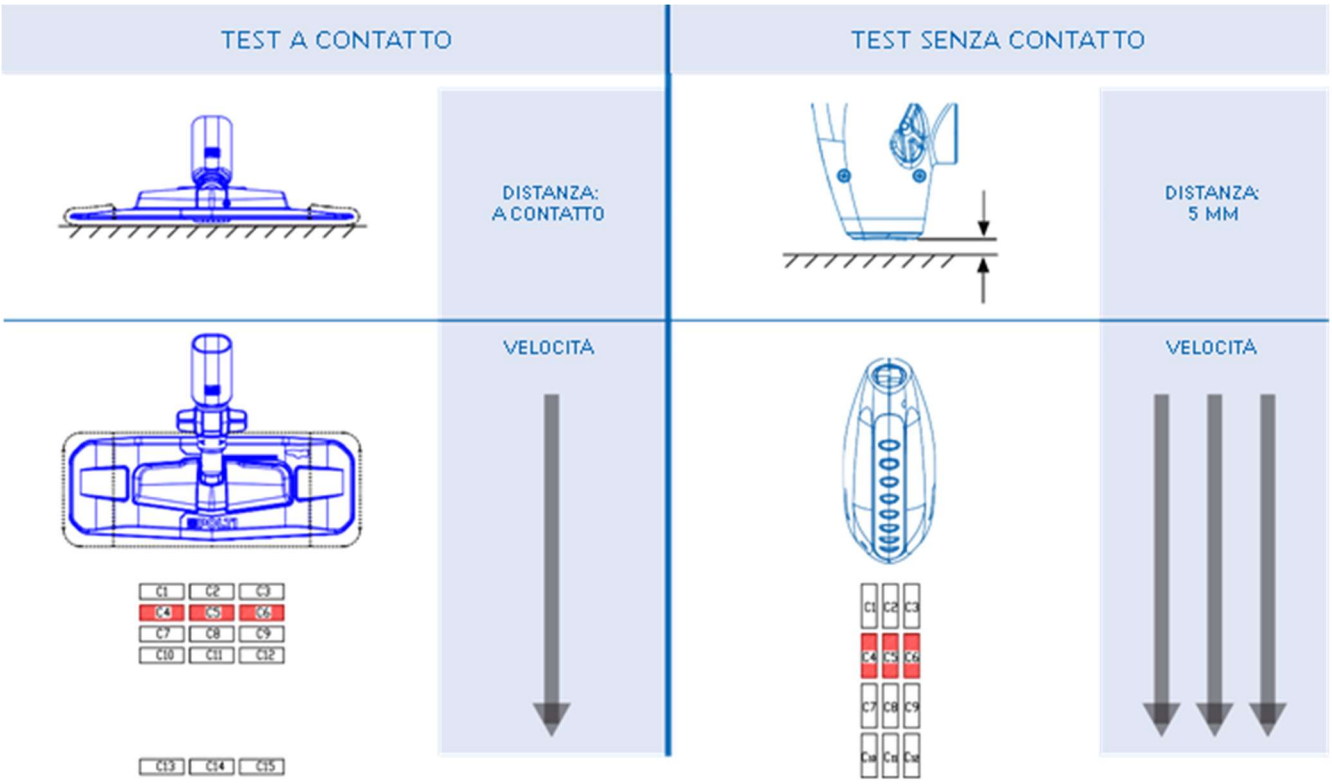
1 ml was taken, in duplicate, from the mixture and its dilutions and placed in Petri dishes to perform an inclusion count for the bacteria in TSA respectively.

After incubation at 36 °C ± 1 °C for 48 h ÷ 96 h, the number of colony-forming units/plate for each dilution, expressed in cfu/ml, was then determined, verifying the non-toxicity of the neutralising agent used for the *surface* antimicrobial efficacy test.

6. BACTERICIDAL ACTIVITY METHOD AFNOR T72-110

The test was carried out only taking into consideration the bactericidal activity on Staphylococcus Aureus applied in the food area. The activity was carried out both with mechanical action and without mechanical action. With mechanical action, i.e. by contact, the test was carried out with a passage speed of 10 cm/s over a surface area of 75 cm^2 , without mechanical action the nozzle must be placed at a distance of 5 mm with a passage speed of 10 cm/s, again testing a surface area of 75 cm^2 .

With mechanical action / with contact | **Without mechanical action / without contact**



Materials and Reagents

Culture medium

For storage of bacterial strains and determination of the viable unit count.

For bacteria

Tryptone Soy Agar (TSA) Composition: Tryptone, pancreatic digestion of casein 15.0 g; Soy peptones, papain digestion of soybean meal 5.0 g; NaCl 5.0 g; agar 15.0 g in 1000 ml pyrogen-free distilled water. Sterilisation in autoclave. pH control at 7.2 ± 0.2 at 20 ± 1 °C.

Diluent

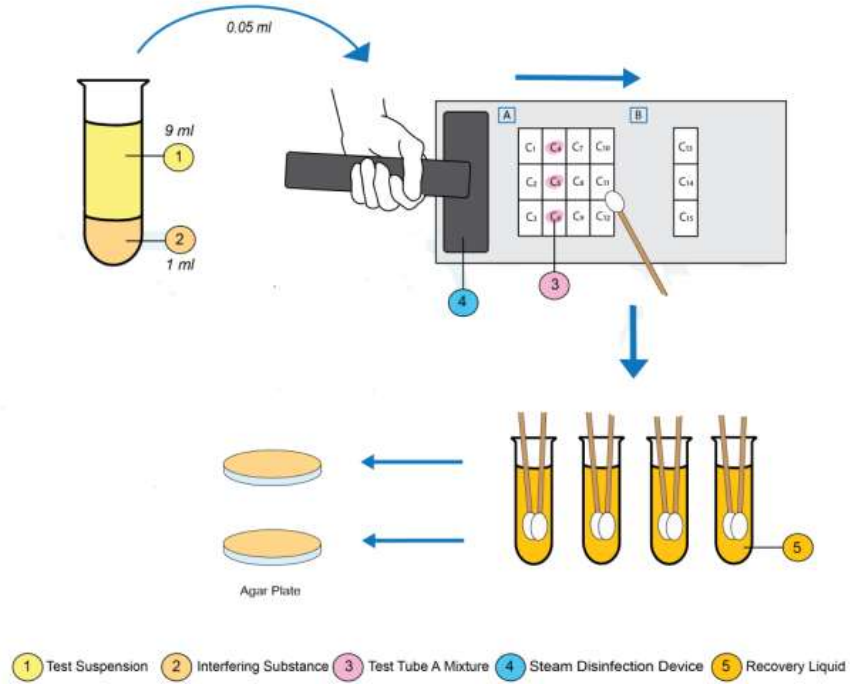
Sodium chloride triptone solution consisting of:
NaCl 8.5 g; Tryptone, pancreatic digestion of casein 1.0 g in 1000 ml pyrogen-free distilled water. pH control = 7.0 ± 0.2 at 20 °C. Autoclave sterilisation.

Neutraliser

The neutraliser has been validated for the tested product.
Composition: Lecithin 10 g/l; Polysorbate 80 50 g/l; Sodium thiosulphate 10 g/l; L-Histidine 0.25 g/l; Phosphate buffer 0.0025 ml/l1 g/l and distilled water at 1000 ml. pH control at 7.0 ± 0.2 at a temperature of 20 ±1°C. Autoclave sterilisation

TEST PROCEDURE

The micro-organism, 50 µl of a known titre 10^7 , is inoculated at point C_4 , C_5 e C_6 and after action of the dry steam dispensing system recovery with surface buffer is carried out. Swabbing is carried out at each point on the surface that has undergone steam dispensing and then returned to solution in a suitable diluent. Subsequently, the logarithmic reduction caused by the system under test is assessed by sampling in a plate.



Calculation of Viability Reduction

For each test microorganism and test concentration of the product, the reduction in live cells was calculated as follows

$$R = \frac{N_w}{N_a}$$

where

- R = reduction in viability
- N_w = test mixture count
- N_a = test mixture count at the end of the contact time

The reduction ($R = N_w / N_a$) is expressed as a logarithm for each micro-organism.

The number of cfu/ml in the test mixture N_w is therefore recorded.

For each product concentration and each experimental condition, the decimal logarithmic reduction was calculated and recorded using the equation

$$\log R = (\log N_w - \log N_a)$$

TEST REPORT

UNI EN 13697:2015 + A1:2019
QUANTITATIVE SURFACE TESTING FOR THE ASSESSMENT OF BACTERICIDAL ACTIVITY IN FOOD, INDUSTRIAL, HOUSEHOLD AND INSTITUTIONAL SETTINGS.

Test method and requirements (Step 2 Step 2)

Laboratory

Laboratory of Microbiology and Chemistry SANA srl Corso Lazio 17 03100 Frosinone Accredited ACCREDIA n°0747

Formulation

System name: ATLANTIS X - Model GV2026 continuous refilling

Composition: Dry steam at 185°C

Manufacturer

EV INTERNATIONAL LTD

KOSTUR 14 A

1618 SOFIA (BULGARIA)

Experimental conditions

- Test date: 24/06/2026- 26/06/2026
- Temperature: 20°C ± 1°C.
- Interfering substances: clean conditions 0.3 % bovine serum albumin and dirty conditions 3.0 % bovine serum albumin + skimmed milk 1/20
- Dispensing times: 5 seconds, 20 seconds
- Instructions for using the device: switch on the machine and wait for it to heat up to 185°C, set speed 2, connect the chosen accessory (in this case the lance), draw off a small amount of steam until an even dispense is achieved. Then approach the accessory at a distance of 5-10 mm and dispense the steam by making continuous wrist movements from right to left and from left to right for the tested dispensing time.

Conclusions

Based on the data obtained in the present study and the criteria of UNI EN ISO13697:2015 + A1:2019, dry steam dispensed for 5 seconds and 20 seconds was shown to induce, under clean and dirty conditions a greater than 4 ***log*** reduction in the viability of the counts under test for bactericidal activity.

Attached Validation Report and Controls

Laboratory Manager



TEST REPORT

UNI EN 13697:2015 + A1:2019
QUANTITATIVE SURFACE TESTS FOR THE EVALUATION OF YEASTICIDAL/FUNGICIDAL ACTIVITY IN FOOD, INDUSTRIAL, DOMESTIC AND INSTITUTIONAL SETTINGS.

Test method and requirements (Step 2 Step 2)

Laboratory

Laboratory of Microbiology and Chemistry SANA srl Corso Lazio 17 03100 Frosinone Accredited ACCREDIA n°0747

Formulation

System name: ATLANTIS X- Model GV2026 continuous refilling

Composition: Dry steam at 185°C

Manufacturer

EV INTERNATIONAL LTD

KOSTUR 14 A

1618 SOFIA (BULGARIA)

Experimental conditions

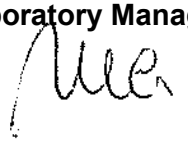
- Test date: 24/06/2026 - 26/06/2026
- Temperature: 20°C ± 1°C.
- Interfering substances: clean conditions 0.3 % bovine serum albumin and dirty conditions 3.0 % bovine serum albumin + skimmed milk 1/20
- Dispensing times: 5 seconds, 20 seconds
- Instructions for using the device: switch on the machine and wait for it to heat up to 185°C, set speed 2, connect the chosen accessory (in this case the lance), draw off a small amount of steam until an even dispense is achieved. Then approach the accessory at a distance of 5-10 mm and dispense the steam by making continuous wrist movements from right to left and from left to right for the tested dispensing time.

Conclusions

On the basis of the data obtained in this study and the criteria of UNI EN ISO 13697:2015 + A1:2019, dry steam dispensed for 5 seconds and 20 seconds was shown to induce, under clean and dirty conditions, a greater than 4 ***log*** reduction in the viability of the counts under investigation for yesticidal and fungicidal activity.

Attached Validation Report and Controls

Laboratory Manager



TEST REPORT

AFNOR T72-110 (SDD) STEAM DISINFECTION DEVICE

Laboratory

Laboratory of Microbiology and Chemistry SANA srl Corso Lazio 17 03100 Frosinone Accredited ACCREDIA n°0747

System name:	ATLANTIS X - Model GV2026 continuous refilling
Composition:	Dry steam at 185°C
Target microorganism:	Staphylococcus aureus ATCC 6538

Manufacturer

EV INTERNATIONAL LTD
KOSTUR 14 A
1618 SOFIA (BULGARIA)

Experimental conditions

- Test date: 24/06/2026 - 26/06/2026
- Temperature: 20°C ± 1°C.
- Interfering substances: clean conditions 0.3 % bovine serum albumin and dirty conditions 3.0 % bovine serum albumin + skimmed milk powder diluted 1/20
- Dispensing speed: 10 cm/sec

Conclusions

Based on the data obtained in the present study and the criteria of AFNOR T72-110, dry steam dispensed at a rate of 10 cm/s was shown to induce a ***5 log*** reduction in the viability of the test counts for bactericidal activity under dirty conditions.

Attached report results

Laboratory Manager



REPORT RESULTS

Strain = Staphylococcus Aureus ATCC 6538 cfu/ml

Test suspension = 3.0 x 10^7

Seed in 50 µl on TSA = 3200 CFU

Summary table of activities and results with different applicators on clean conditions AFNOR T72-110

APPLICATOR	Logarithmic reduction	Percentage reduction	TIPOLOGY of ACTION
	R = >5	99.999 %	With contact
	R = >5	99.999 %	With contact
	R = >5	99.999 %	Without contact
	R = >5	99.999 %	Without contact

Laboratory Manager

Summary table of activities and results with different applicators on dirty conditions AFNOR T72-110

APPLICATOR	Logarithmic reduction	Percentage reduction	TIPOLOGY of ACTION
	R = >5	99.999 %	With contact
	R = >5	99.999 %	With contact
	R = >5	99.999 %	Without contact
	R =>5	99.999 %	Without contact

Laboratory Manager