EVALUATION OF THE BIOCIDAL ACTIVITY OF A "PROTECT" AIR FRESHENER

ACCORDING TO A METHOD INSPIRED BY STANDARD NF EN 17272: 2020

ON *ASPERGILLUS BRASILIENSIS*

(20°C ± 2°C – CLEAN CONDITIONS)

Report written by: Melanie BAROU

Aix en Provence on 08/31/2022

CHANGE HISTORY

Each revision of the report cancels and replaces the previous one.

In order to limit the risk of errors and misuse of outdated documents, any canceled report as well as the copies must be destroyed.

Change history		
Report No.	Paragraph(s) amended	Reason for the modification
3465.GTS.22	-	Report creation

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I. DESCRIPTION OF THE STUDY:

Entitled :	Evaluation of the biocidal activity of a "PROTECT" air freshener according to a method based on standard NF EN 17272: 2020 on*Aspergillus brasiliensis*(20°c ± 2°C – Clean conditions)
Internal code:	Study No.: 3465.GTS.22
Sponsor:	GPS MONACO GROUP 5 rue Gabian The Triton - 5th floor 98000 Monaco Contact: Nicolas Blasin
Study period:	From: 08/10/2022 to 08/16/2022
Responsible for the study:	Melanie BAROU
Performing tests :	Ebru SELVITOP
Test laboratory:	EUROFINS BIOTECH Laboratory – GERMANDE 505 rue Louis Berton Building 2 13290 Aix-en-Provence

II. GOAL OF THE STUDY :

Evaluate, according to a method inspired by standard NF EN 17272, the "PROTECT"
to be reduced, in the presence of interfering substances (conditions of at least
pr 10⁴the number of viable cells*Aspergillus brasiliensis*stainless dep. are steel

III. DEVICE TESTED:

Last name :	PROTECT (see figure
Serial number*:	n°1) Not disclosed
Manufacturer:	GPS Monaco Group
Technology used*:	cold plasma

* Data provided by the customer, cannot engage the responsibility of the laboratory.

Figure 1:PROTECT►

IV. METHOD :

a) Strain tested

*Aspergillus brasiliensis*ATCC 16404

The storage and control conditions for the fungal strain used to determine the fungicidal activity are those described in European standard NF EN 12353 (internal protocol: T-DM-S-W037879).

b) Interfering substance

Bovine albumin:	0.03g
Tryptone-salt:	qsp 100ml
Bovine albumin concentration :	0.3g/l
Internal code:	149AA00416

Sterilized by membrane filtration.

c) Neutralizing

Composition of the neutralizer:

Tween 80:.....	10% (v/v)
Lecithin:.....	2%
Sodium thiosulphate: .... L-	2%
Histidine:	2%
Saponin:	1%
Trypticase soy broth:	qsp 100ml

Sterilized in autoclave (121°C, 21 minutes).

Internal code: E586.1.2, E592.1.1

d) Interview and enumeration environment

fungal agar

Sterilized in autoclave (121°C, 21 minutes).

Internal code: E587.1.1, E587.1.2, E587.1.3, E587.1.4

e) Media

Stainless steel discs, flat corresponding to the requirements of paragraph 5.2.3.2 of the standard.

f) Conditions of use of the device

The method used for the tests is that described by standard EN NF 17272 (see Fig. 2). The only parameter that has changed is the distance between the test supports and the device under test.

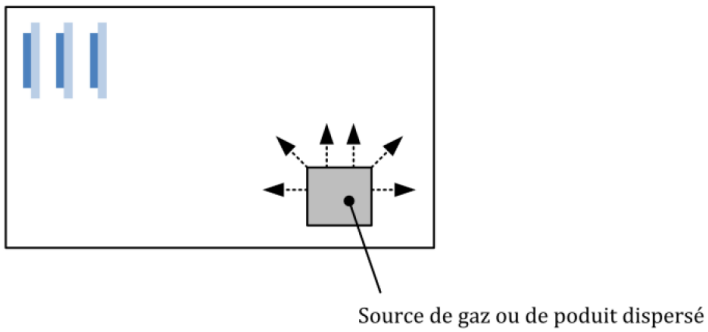

Figure 2. Arrangement of equipment during the test. The supports are placed facing away from the disinfection process according to the specifications of the test standard.

Volume of the test piece:.....	34.5m3
Distance supports-device tested:	1m
Temperature at the start of the tests:	20°C ± 2°C
Incubation temperature:	30°C ± 1°C
Relative humidity at the start of the tests :	Between 50 and 75%

V. RESULTS

Time to contact	Suspension microbial trial (NOT) (No. CFU/ml)	Preliminary tests			Witness positive (T) (Nb. CFU/support)	Trials		
		not1/NOT1	not2/NOT2	not3/NOT1		n'1+n'2	Reduction medium (Log10)	Red %
12 hours	3.6.10$_7$	2.3	0.9	1.8	$T1_{2\ hours}$=4.0.10$_5$	2.8.10$_5$	$R_{average}$= 0.1	29%
24 hours					$T_{24\ hours}$=2.8.10$_5$	2.3.10$_5$	$R_{average}$= 0.1	16%

Table 1: Results. Evaluation of the biocidal activity of the "PROTECT" process according to a method inspired by standard NF EN 17272 with respect to*Aspergillus brasiliensis*ATCC 16404. T: number of microorganisms on control disc. N1: count of the test suspension by dilution/inclusion- N2: count of the test suspension by filtration. n1: Search for an inhibitory effect in the agar medium – n2: Search for an inhibitory effect in the membranes. n'1: number of test organisms (or plaques) surviving in 100 ml of recovery liquid – n'2: number of colonies obtained directly by inoculation by inclusion of the test medium. n'1+n'2: number of microorganisms on the test medium. d1: disc N°1/ d2: disc N°2/ d3: disc N°3. The experimental conditions are validated if n1/N1 > 0.5, n2/N2 > 0.5 and n3/N1 > 0.5, meaning that neither the culture medium, nor the disc, nor the filtration membrane have any inhibitory effect.

VI. FINDINGS

Under the experimental conditions described, the PROTECT device leads to an average reduction in the number of viable cells of*Aspergillus brasiliensis*ATCC 16404 deposited on 0.1 log stainless steel supports$_{10}$after a contact time of 12 and 24 hours in the presence of interfering substances (clean conditions).

VII. REFERENCES

1-NF EN 17272: April 2020. Chemical antiseptics and disinfectants - Methods of disinfection of parts by air using automated processes - Determination of bactericidal, fungicidal, yeasticidal, sporicidal, tuberculocidal, mycobactericidal, virucidal and phagocidal activity.

2- Guide to good practices for disinfection of medical devices. Superior Council of Public Hygiene of France.https://www.vie-publique.fr/rapport/24373-guide-de-bonnes-pratiques-dedesinfection-des-dispositifs-medicaux

VIII. GOOD LABORATORY PRACTICES

This study was carried out in accordance with the general requirements of standard NF EN ISO/IEC 17025 (2017) relating to the skills of calibration and test laboratories. Standard operating procedures and good laboratory practices were followed in this study.

The original data of this report, the workbooks, the protocols and the final study report are stored in the archives of Eurofins Biotech-Germande under the reference 3465.GTS.22.

Melanie BAROU

On 08/31/2022

EVALUATION OF THE BIOCIDAL ACTIVITY OF THE AIR PURIFIER « PROTECT » ACCORDING TO THE EN 17272:2020 STANDARD AGAINST *STAPHYLOCOCCUS AUREUS*

(20°C ± 2°C – CLEAN CONDITIONS)

Report written by: Mélanie BAROU

Aix-en-Provence 04/11/2022

VERSION HISTORY

Each revision of the report cancels and supersedes the previous one.
In order to limit the risk of misuse of outdated documents, any cancelled reports and copies should be destroyed.

Version history		
Report N°	Amended paragraphs	Purpose of the modification
3371.GTS.22	-	Initial version

TABLE OF CONTENTS

I. STUDY DESCRIPTION:

Title:	Evaluation of the biocidal activity of the air purifier « PROTECT » according to the NF EN 17272:2020 standard (20°c ± 2°C – Clean conditions)
Internal number:	Study N°: 3371.GTS.22
Sponsor:	GPS MONACO GROUP 5 rue Gabian Le Triton - 5ème étage 98000 Monaco Contact: Nicolas Blasin
Study period:	16/06/2022 - 21/06/2022
Study manager:	Mélanie BAROU
Technicians:	Méryl JAONINA / Ebru SELVITOP
Test laboratory:	Laboratoire EUROFINS BIOTECH – GERMANDE 505 rue Louis Berton Bâtiment 2 13290 Aix-en-Provence

II. OBJECTIVE:

Evaluate, according to the conditions described in the NF EN 17272 standard, the ability of the device « PROTECT » to reduce, in presence of interfering substances (clean conditions), in 2h, 6h and 24h, of at least 10^5 the number of viable cells of *Staphylococcus aureus* inoculated on stainless steel discs.

III. TESTED DEVICE:

Name: ……	PROTECT (figure n°1)
Batch number* : ……	Not provided
Manufacturer : ……	GPS Monaco Group
Technology* : ……	Cold plasma

*Data provided by the customer, do not engage the responsibility of the laboratory

Figure n°1: PROTECT ►

IV. METHOD:

a) Tested strain

Staphylococcus aureus CIP 4.83

The conditions of conservation and control of the bacterial strain used for the determination of the bactericidal activity are those described in the NF EN 12353 standard (internal protocol: T-DM-S-W037879).

b) Interfering substance

Bovine albumin:	0.03g
Tryptone salt:	q.S.p. 100ml
Bovine albumin concentration:	0.3g/l
Internal reference:	149AA00416

Sterilized by membrane filtration.

c) Neutralizing solution

Tween 80 :.....	10% (v /v)
Lecithin :.....	2%
Sodium thiosulfate :	2%
L-Histidine :	2%
Saponin :.....	1%
Trypticase soya broth:	q.s.p. 100ml

Sterilized by autoclave (121°C, 21 minutes).

Internal references: E557.1.1/ E491.1.2/ E555.1.1

d) Counting medium

Trypticase Soya Agar

Sterilized by autoclave (121°C, 21 minutes).

Internal references: E550.1.1/ E550.1.4/ E550.1.3/ E550.1.1

e) Supports

Stainless steel discs, in accordance with requirements of the paragraph 5.2.3.2 of the standard.

f) Conditions of use of the device

The test method used is the one described in the NF EN 17272 standard (Fig. 2).

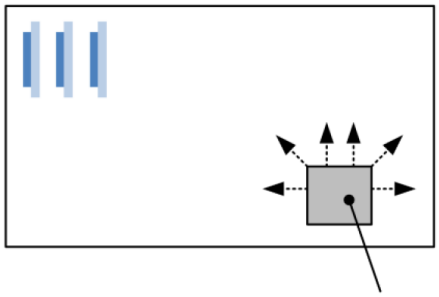

Figure 2. Equipment set up for the tests. The supports are placed opposite to the device according to the requirements of the standard.

Volume of the test chamber :.....	34.5 m^3
Temperature at the beginning of the tests :	20°C ± 2°C
Incubation temperature :	37°C ± 1°C
Relative humidity at the beginning of the tests :	50%-75%

V. RESULTS

Contact times	Microbial test suspension (N) (Nb. CFU/ml)	Preliminary tests			Positive control (T) (Nb. CFU/support)	Assays		
		n_1/N_1	n_2/N_2	n_3/N_1		n'1+n'2	Mean reduction (Log10)	Red %
2 hours	$1,3.10^8$	4.1	1.9	1.6	$T_{2h}=3,9.10^6$	$2,1.10^6$	**R_{moy} = 0,3**	**47%**
6 hours					$T_{6h}=4,1.10^6$	$6,7.10^5$	**R_{moy} = 0,8**	**83%**
24 hours					$T_{24h}=2,3.10^6$	$9,8.10^4$	**R_{moy} = 1,4**	**96%**

Table 1 : Results. Evaluation of the biocidal activity of the device « PROTECT » according to the NF EN 17272 standard against *Staphylococcus aureus* CIP 4.83. T : number of microorganisms on the control disc. N1 : enumeration of the test suspension by dilution/inclusion- N2 : enumeration of the test suspension by filtration. n1 : inhibitory effect in the agar medium – n2 : inhibitory effect of the membranes. n'1 : number of surviving microorganisms in 100 ml of the neutralizing solution – n'2: number of colonies obtained by direct inclusion of the disc. n'1+n'2 : number of microorganisms per disc. d1 : disc N°1/ d2 : disc N°2/ d3 : disc N°3. Experimental conditions are validated if n1/N1 > 0.5, n2/N2 > 0.5 et n3/N1 > 0.5, if neither the counting agar, the membranes, nor the supports have any inhibiting effects. CFU: colony forming unit.

VI. CONCLUSIONS

Considering the experimental conditions described, the device « PROTECT » is able to reduce of 1.4 $\log_{10}$ (i.e. 96% of the initial inoculum) the number of viable cells of *Staphylococcus aureus* CIP 4.83 inoculated on stainless steel discs, after a contact time of 24 hours in presence of interfering substances (clean conditions).

VII. REFERENCES

1-NF EN 17272: April 2020. Chemical disinfectants and antiseptics – Methods of airborne room disinfection by automated process – Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities.

VIII. GOOD LABORATORY PRACTICES

This study was conducted according to NF EN ISO/IEC 17025 (2017) General requirements for the competence of testing and calibration laboratories. Applicable standard operating procedures and good laboratory practices were followed in this study.

The original records of this report, the notebooks and protocol are stored in the archives of Eurofins Biotech Germande “3371.GTS.22”.

Melanie BAROU
Le 04/11/2022