



---

Study Report	Evaluation of Sporicidal Activity of Disinfectants Standard EN13704:2018
Reference	AB01220425_v02
Identification of the Sponsor	Success Gadget, Nanotecnologia e Novos Materias, Lda Rua Filipa Borges, 1245 4750-823 Barcelos
Sample identification	CaresUs, desinfetante de superfícies com base na tecnologia Si2U de forma eficaz e duradoura Batch AMS220203
Sampling	By the sponsor
Type of sample	Biocide, Liquid Plastic - 250mL
Date of sample reception	28/01/2022

---

Covilhã, 31<sup>th</sup> March 2022

---

Study director  
(Carlos Gaspar, MSc, Biochemistry)



## Document history

---

Version	Alterations	Date
v_01	First version of the document	10-03-2022
v_02	Second version of the document: Change of sample identification	31-03-2022

## Proponent and Test Facilities identification

---

Proponent	Labfit - HPRD: Health Products Research and Development Lda
Address	Edifício UBIMEDICAL, Estrada Municipal 506, 6200-284 Covilhã
Responsible for the quality management system	Lígia Borges, MSc, Chemist
Study director	Carlos Gaspar, MSc, Biochemistry
Technicians	Joana Raimundo Catarina Fernandes

## Quality Management System

---

Labfit's quality assurance program is ensured by periodic audits and inspections of the quality management systems implemented: ISO 9001: 2015, ISO 13485: 2016, NP 4457: 2007 and Good Laboratory Practice (GLP) pursuant to Directive 2004/9 / EC. The last GLP inspection took place on 22, 23 and 24/05/2018 and the last external audit took place on 25, 26 and 27/08/2021.

The results hereby reported reflect the data registered during the study made for the tested sample.

**The information included within this report is confidential and will not be disclosed, fully or partially, without the previous consent from the sponsor.**

## Test principle

---

The test was carried out according to standard EN 13704: 2018 - Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1).

To a product sample tested directly or diluted in hard water (or water for ready-to-use products) is added a bacterial spore's suspension in a solution with an interfering substance. The mixture is maintained at the temperature and contact times defined in the standard. After the contact time, an aliquot is removed, and the sporicidal activity is immediately neutralized or suppressed through a validated method. The number of surviving bacterial spores recovered is determined, and the reduction is calculated.

## Study conditions

---

Study beginning date	14/02/2022
Test beginning date	14/02/2022
Test conclusion date	17/02/2022
Study conclusion date	10/03/2022
Sample storage during the test	At room temperature in the package sent by the sponsor.



## Materials

---

### Reagents

---

Culture medium	Tryptone Soya Agar (TSA)
Neutralizer	Buffered peptone water 20g/L added with tween 80 30g/L (Prolabo), soy lecithin 3g/L (Prolabo), saponins 30g/L (Prolabo) and triton 1g/L (Prolabo)
Interfering substabce	BSA (VWR) 0.3 g/L
Water distilled	Ultrapure Type 1, Ditect-Q 3UV

---

### Test microorganism and conditions of incubation

---

Microorganism	<i>Bacillus subtilis</i> ATCC 6633; 30°C, 72 hours
---------------	--

---



## Equipment

Precision Balances	VWR, SE 1202 (LAB010); VWR, LPC-503i (LAB027)
Autoclave	A.J. Costa, LDA., Uniclave 88 40x60 (LAB055)
Water baths	VWR, 462-0355 (LAB026); Memmert, WB 29 (LAB150)
Incubator	Memmert, ICP 500 (LAB180)
Refrigerators	Electronia, HS-121LN (LAB083); Zanussi, ZRA40401WA (LAB133); AEG, 72348-KA (LAB171)
Magnetic stirrer and heating plate	Heidolph, MR Hei-Standard (LAB030)
pH meter: semi-solid	Testo, 206-pH2 (LAB115)
Shaker	Velp Scientifica, TX4 Digital IR Vortex Mixer (LAB056)
Spectrophotometer UV/VIS	VWR, UV-3100 PC (LAB011)
Purified Water System	Merck, Direct Q @3UV (LAB059); Aquawin (LAB234)
Micropipettes	VWR, VE1000 (LAB007); VWR, VE5000 (LAB032); VWR, VE10000 (LAB033)
Stopwatch	VWR, bioMarke 609-0217 (LAB091)
Centrifuge	Thermo Scientific, Heraeus Megafuge 8 (LAB023)
Colony counter	Stuart, SC6 Plus (LAB050)
Dataloggers	Ebro, EBI 20 T1 (LAB065); Ebro, EBI 20 TI (LAB088); Ebro, EBI 20 (LAB182); Ebro, EBI 20 (LAB289)
Bunsen burner	Electrothermal, BA6101 (LAB119)
Equipment	Usual laboratory equipment

## Methods

The test was carried out at a temperature of  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and the contact time was 60 minutes, using the microorganism described in the previous point. The techniques used were dilution-neutralization, plate count, and inoculation by incorporation, in duplicate.

The conditions regarding the time and temperature used were agreed with the promoter.

The product was tested at concentrations of 80% (v/v), 8% (v/v), and 0.08% (v/v) after dilution in water.

### Validation of test conditions for the sample under study

The validation of the test conditions for the sample under study and the verification of the absence of toxicity in the test conditions were carried out during the test and according to the standard's indications.

### Assay criteria

According to the standard in reference, the classification of a product with sporicidal activity is dependent on, after the fulfilment of the test conditions, a reduction of at least 3 logs in relation to the initial concentration of the inoculum in the sample.

The test criteria indicated in the EN 13704 standard are described in table 1.

**Table 1.** Test criteria applied to controls N, N<sub>0</sub>, N<sub>v</sub>, N<sub>v0</sub>, A, B and C for the microorganism tested.

Microorganism ( <i>Bacillus subtilis</i> )	Criteria (EN13704:2018)	
	CFU/mL	log(N)
Initial suspension (N)	$1.5 \times 10^6 - 5.0 \times 10^6$	6.17 – 6.70
Weighted average control	5 - 15	----
Initial suspension (N <sub>0</sub> )	$1.5 \times 10^5 - 5.0 \times 10^5$	5.17 – 5.70
Suspension of validation (N <sub>v</sub> )	$3.0 \times 10^2 - 1.6 \times 10^3$	----
Sample suspension (N <sub>v0</sub> )	30-160	----
Validation of test conditions (A)	$\geq 0.5 N_{v0}$	----
Control of the Neutralizer's absence of toxicity (B)	$\geq 0.5 N_{v0}$	----
Validation control of dilution neutralization (C)	$\geq 0.5 N_{v0}$	----

## Test Results

### Validation of test conditions for the sample under study

**Table 2.** Concentration of microorganism in the working suspension (initial suspension) and in the sample after inoculation (Controls N and N<sub>0</sub>).

Microorganism	Initial suspension (N)		Conformity	Concentration of the suspension in the sample (N <sub>0</sub> )		Conformity
	(CFU/mL)	log(N)		(CFU/mL)	log(N <sub>0</sub> )	
<i>Bacillus subtilis</i>	3.31x10 <sup>6</sup>	6.52	Yes	3.31x10 <sup>5</sup>	5.52	Yes

**Table 3.** Control of the weighted average in the calculation of N.

Microorganism	Results	Conformity
<i>Bacillus subtilis</i>	8	Yes

**Table 4.** Results obtained for the controls N<sub>v</sub>, N<sub>v0</sub>, A, B and C included in the assay.

Microorganism	Suspension of validation N <sub>v</sub>	Suspension of validation N <sub>v0</sub> (CFU/mL)	Control A (CFU/mL)	Control B (CFU/mL)	Control C (CFU/mL)
<i>Bacillus subtilis</i>	1.05x10 <sup>3</sup>	105	141	116	75

## Test results for the product under test

The experimental results obtained are shown in table 5.

**Table 5.** Counts of CFU/mL and respective logarithmic reduction in the various product concentrations tested.

Microorganism	CaresUs, desinfetante de superfícies com base na tecnologia Sil2U de forma eficaz e duradoura 80%		CaresUs, desinfetante de superfícies com base na tecnologia Sil2U de forma eficaz e duradoura 8%		CaresUs, desinfetante de superfícies com base na tecnologia Sil2U de forma eficaz e duradoura 0.08%	
	CFU/mL	Log Reduction	CFU/mL	Log Reduction	CFU/mL	Log Reduction
<i>Bacillus subtilis</i>	<1.40x10 <sup>2</sup>	>3	>3.30x10 <sup>3</sup>	<2	>3.30x10 <sup>3</sup>	<2

## Discussion of the results and conclusions

The results presented in tables 2, 3 and 4 show the fulfilment of all the validation criteria indicated in the standard EN 13704 for the microorganism *Bacillus subtilis*.

In table 5, the product "CaresUs, desinfetante de superfícies com base na tecnologia Sil2U de forma eficaz e duradoura, batch AMS220203" shows a log reduction of at least 3 logs, using BSA at 0.3 g/L as an interfering substance, after contact for 60 minutes, at 20°C. The concentration of 80% (v/v) is active for the microorganism; and 8% (v/v) and 0.08% (v/v) is non-active.

Thus, it is concluded that the product under test has sporicidal activity against the microorganism studied, under conditions suitable for its use, at a concentration of 80% (v/v) relative to the concentration in the original packaging.

## Study records storage and general data storage

All records related to the study (study plan, raw data, spreadsheets and report) will be kept in the file, at Labfit facilities.