



**ASSESSMENT OF BASIC FUNGICIDE ACTIVITY OF ANTISEPTICS
AND CHEMICAL DISINFECTANTS USED IN FOOD PRODUCTS, IN
THE INDUSTRY, IN THE HOME AND IN COLLECTIVES**

Test method by dilution-neutralization
(UNE-EN 1275 Standard of June 2006)

CLIENT

PERSEIDA BELLEZA, S.A.
Poligono Industrial El Pabellon S/N
06380 JEREZ DE LOA CABALLEROS (BADAJOZ)

LABORATORIO MICRO

LABORATORIO MICRO-BIOS, S.L.
Av. Mossen Cinto Verdaguer, 62
Poligono Fontsa
08970 SANT JOAN DESPI (BARCELONA)



This European Standard specifies a test method and the minimum requirements to verify the basic fungicidal activity of antiseptic or disinfectant chemicals that form a homogeneous preparation that is physically stable in water and that are used in agriculture (but not for crop protection), domestic service, food hygiene and other industrial, institutional, medical and veterinary fields.

This document indicates the experimental conditions being carried out simultaneously and the validation of the test method by dilution neutralization indicated in the Standard.

The two tables are attached, Table I with the results of the validation under the established conditions and Table II with the results obtained.

Based on the results obtained, a conclusion is drawn up where the applied Standard is indicated, the description of the sample with the experimental conditions and an opinion always in accordance with the indications in the conclusions section of the Standard.



1. IDENTIFICATION OF THE TEST CENTER

Center: LABORATORIO MICRO-BIOS, S.L (Dept. of Microbiology)
Address: Av. Mossèn J. Verdaguer, 62-P.I. Fontsanta
08970 ST. JOAN DESPI (BARCELONA)
Technical manager: Dr. Carlos A. Santamaria Rojas

2. CLIENT IDENTIFICATION

Requested by: PERSEIDA BELLEZA, SA
Address: Poligono Industrial El Pabellón S / N Population: 06380
Population: JEREZ DE LOS CABALLEROS (BADAJOZ)

3. IDENTIFICATION OF THE SAMPLE

Product name: HIDROALCOHOLIC GEL DISINFECTANT OF HANDS SAMPLE 2
Batch number: Not indicated
Expiration: Not indicated
Manufacturer or Supplier: PERSEIDA BELLEZA, S.A.
Active substance: Ethyl alcohol 96 (62%)
Isopropyl alcohol (10%)
Storage condition: 0 – 40 °C
Appearance of the product: Colourless gel
Sample taken (*): Handed over the stakeholders
Internal reference: 1286.02-09

(*) Sampling is not covered by the scope of ENAC accreditation

Page 3 of 7

4. RELATED DATES

CERTIFICATE
UNE-EN ISO
9001.2000
Nº CS/00/043

ACCREDITED
UNE-EN ISO
17025.2005
Nº 546/LE1162

Avda. Mossen Cinto Verdaguer, 62-Poligono Industrial Fontsanta
– 08970 Sant Joan Despi (Barcelona) Tel. 93 477 18 27
Fax. 93 373 09 60 – E-mail: info@laboratoriomicro-bios.com
C.I.F.B-58278334

CERTIFICATE
BPL
Nº 031CAT

CERTIFICATE
NCF/GMP
Nº 0736/001/CAT



Deadline: 7th Sep 2009
Test start date: 9th Sep 2009
Test end date: 18th Sep 2009
Report release date: 21st Sep 2009

5. TEST METHOD

UNE-EN 1275 (06/2006): Basic fungicide activity of antiseptics and disinfectants chemicals. Neutralization dilution test method (phase 1).

6. EXPERIMENTAL CONDITIONS

- 6.1. Product concentrations tested: 80% (v / v)
- 6.2. Contact time: 1 minute 10 s.
- 6.3. Test temperature: 20 ° C ± 1 ° C.
- 6.4. Product diluent: Sterile water for injections.
- 6.5. Incubation temperature: 30 ° C ± 1 ° C.
- 6.6. Strains used:
 - Aspergillus niger ATCC 16404
 - Candida albicans ATCC 10231
- 6.7. Thinner-neutralizer used: 0.1% peptonised physiological serum with:
 - Tween 80 to 10%
 - Lecitina 6%
 - Sodium Dodecyl Sulfate 1%
- 6.8. Culture media used:
 - Malta Extract Agar (MEA)
- 6.9. Appearance of product dilutions: Colorless gel
- 6.10. Storage conditions: Laboratory temperature (≈20 ° C)



7. RESULTS: See attached tables: I and II

8. Special observations

To declare a basic fungicidal activity, the European standard EN 1275 requires a demonstrating a logarithmic reduction of at least 4 ($\geq 1.0 \times 10^4$ ufc/ml) in counts fungal fungi against *Aspergillus niger* and *Candida albicans* under the conditions of established tests ($20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ and 5 min. of contact).

At the request of the client, a single concentration was tested against the two strains required by the standard.

Only one replication has been made per test body.

All controls and validation were within their basic limits.

No precipitate formed during the test procedure (test mixtures were homogeneous).

9. CONCLUSION

According to the tests carried out and partially following the procedure indicated in the UNE-EN 1275 standard (June 2006), sample 1286.02-09, identified as HYDROALCOHOLIC GEL HAND DISINFECTANT SAMPLE 2, when diluted in water for injection at a concentration of 80% (v/v), it has fungicidal activity after 1 minute of contact, if the test organisms are: *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231. The reduction in viability is greater than 4 logarithms ($<1.0 \times 10^4$ ufc/ml) under the specified test conditions.

(Signature)

Sant Joan Despi, 21th September 2009

Carlos A. Santamaria

Ana Vila-Ferran

Responsible Technician

Managing Director

The data in this report is archived for a period of 5 years and the samples delivered 3 months or until their expiration

This report only affects the sample tested

Total or partial reproducibility of this report is prohibited without the express permission of the issuing laboratory.

Page 5 of 7

CERTIFICATE
UNE-EN ISO
9001.2000
N° CS/00/043

ACCREDITED
UNE-EN ISO
17025.2005
N° 546/LE1162

Avda. Mossen Cinto Verdaguer, 62-Poligono Industrial Fontsa
- 08970 Sant Joan Despi (Barcelona) Tel. 93 477 18 27
Fax. 93 373 09 60 - E-mail: info@laboratoriomicro-bios.com
C.I.F.B-58278334

CERTIFICATE
BPL
N° 031CAT

CERTIFICATE
NCF/GMP
N° 0736/001/CAT

TABLE I

Validation of the dilution method of sample 1286.02-09 at 80% (v/v)

ORGANISM OF TEST	COUNT (cfu /ml.)			
	Nv	A	B	C
<i>A. niger</i> ATCC 16404	(129+131) / 2 = 130	(145+149) / 2 = 147	(113+132) / 2 = 122.5	(142+164) / 2 = 153
<i>C. albicans</i> ATCC 10231	(80+83) / 2 = 81.5	(72+80) / 2 = 75	(74+74) / 2 = 74	(84+88) / 2 = 86

Nv = Bacterial Validation Suspension = 30 – 160 cfu / ml

A = Control of the experimental condition ($A \geq 0.5 Nv$)

B = Neutralizer of Filtration Control ($B \geq 0.5 Nv$)

C = Validation of the method ($C \geq 0.5 Nv$)

TABLE II

Test results of sample 1286.02-09 at 80% (v/v) and 1 minute ± 10 s. of contact

ORGANISM OF TEST	COUNT (cfu /ml.)					R	
	N	Nv	A	B	C	Na	
						80%	80%
<i>A. niger</i> ATCC 16404	10 ⁻⁵ :>165;>165	(93+103)	(87+92)	(93+102)	(111+117)	(<14+<14)	
	10 ⁻⁶ : 40; 41	2	2	2	2	2x10 ⁻¹	
	N = 4.0 x 10⁷						
	No = N/10	= 98	= 89.5	= 97.5	= 114	=<140	>4.45
	Lg No = 6.60					LgNa: <2.15	
<i>C. albicans</i> ATCC 10231	10 ⁻⁵ :>300;>300	(134+150)	(156+172)	(159+189)	(119+160)	(<14+<14)	
	10 ⁻⁶ : 48; 50	2	2	2	2	2x10 ⁻¹	
	N = 4.9 x 10⁷						
	No = N/10	= 142	= 164	= 174	= 139.5	=<140	>4.54
	Lg No = 6.69					LgNa: <2.15	

N = Test Fungal Suspension = 1.5 x 10⁷ - 5 x 10⁷ ufc/ml

No = N/10 = 6.17 ≤ Lg No ≤ 6.70

Nv = Fungal Validation Suspension = 30 – 160 cfu / ml

A = Control of the experimental condition (A ≥ 0.5 Nv)

B = Neutralizer of Filtration Control (B ≥ 0.5 Nv)

C = Validation of the method (C ≥ 0.5 Nv)

Na = Viable in 1 ml of the Test Mix

R = Logarithmic reduction = Lg No – Lg Na