



**ASSESSMENT OF BASIC BACTERICIDE 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 1040 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  
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This European Standard specifies a test method and the minimum requirements to verify the basic bactericidal 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



#### 4. RELATED DATES

Deadline: 8<sup>th</sup> Sep 2009  
Test start date: 9<sup>th</sup> Sep 2009  
Test end date: 18<sup>th</sup> Sep 2009  
Report release date: 21<sup>st</sup> Sep 2009

#### 5. TEST METHOD

UNE-EN 1040 (06/2006): Basic bactericidal 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: 36 ° C ± 1 ° C.
- 6.6. Strains used:
  - Pseudomonas aeruginosa ATCC 15442
  - Staphylococcus aureus ATCC 6538
- 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:
  - Tryptonated Soya Agar (TSA)
- 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 bactericidal activity, the European standard EN 1040 requires a demonstrating a logarithmic reduction of at least 5 ( $\geq 1.0 \times 10^5$  ufc/ml) of the viable bacterial counts against *Pseudomona aeruginosa* and *Staphylococcus aureus* 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 1040 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 basic bactericidal activity after 1 minute of contact, if the test organisms are: *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6538. The reduction in viability is greater than 5 logarithms 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.

**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>P. aeruginosa</i> ATCC 15442	(97+121) / 2 <b>= 109</b>	(101+135) / 2 <b>= 118</b>	(101+115) / 2 <b>= 108</b>	(86+122) / 2 <b>= 104</b>
<i>S. aureus</i> ATCC 15442	(82+83) / 2 <b>= 82.5</b>	(84+89) / 2 <b>= 86.5</b>	(79+96) / 2 <b>= 87.5</b>	(86+94) / 2 <b>= 90</b>

**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>P. aeruginosa</i> ATCC 15442	10 <sup>-6</sup> : 217; 221	(62+67)	(77+90)	(60+90)	(74+99)	(<14+<14)	
	10 <sup>-7</sup> : 29; 30	2	2	2	2	2x10 <sup>-1</sup>	
	<b>N = 2.3 x 10<sup>8</sup></b>						
	<b>No = N/10</b>	<b>= 64.5</b>	<b>= 83.5</b>	<b>= 75</b>	<b>= 86.5</b>	<b>=&lt;140</b>	<b>&gt;5.2</b>
	<b>Lg No: 7.35</b>					<b>LgNa: &lt;2.15</b>	
<i>S. aureus</i> ATCC 15442	10 <sup>-6</sup> : 151; 152	(57+58)	(67+82)	(64+68)	(74+76)	(<14+<14)	
	10 <sup>-7</sup> : 15; 19	2	2	2	2	2x10 <sup>-1</sup>	
	<b>N = 1.5 x 10<sup>8</sup></b>						
	<b>No = N/10</b>	<b>= 57.5</b>	<b>= 74.5</b>	<b>= 66</b>	<b>= 75</b>	<b>=&lt;140</b>	<b>&gt;5.03</b>
	<b>Lg No: 7.18</b>					<b>LgNa: &lt;2.15</b>	

**N** = Bacterial Test Suspension = 1.5 x 10<sup>8</sup> - 5 x 10<sup>8</sup> ufc/ml

**IgNo** = lg(N/10) = 7.17 ≤ Lg No ≤ 7.70

**Nv** = Bacterial 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