

# **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)**

The European Standard EN 13704:2002 has the status of a  
British Standard

ICS 71.100.35

# National foreword

This British Standard is the official English language version of  
EN 13704:2002

The UK participation in its preparation was entrusted to Technical Committee  
IH/57, Chemical disinfectants and antiseptics, which has the responsibility to:

- aid enquirers to understand the text;
- present to the responsible European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
- monitor related international and European developments and promulgate them in the UK.

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English version

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)

Désinfectants chimiques - Essai quantitatif de suspension  
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Suspensionsversuch zur Bestimmung der sporiziden  
Wirkung chemischer Desinfektionsmittel in den Bereichen  
Lebensmittel, Industrie, Haushalt und öffentliche  
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This European Standard was approved by CEN on 11 November 2001.

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CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.



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## Foreword

This European Standard has been prepared by Technical Committee CEN/TC 216 "Chemical disinfectants and antiseptics", the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2002, and conflicting national standards shall be withdrawn at the latest by August 2002.

In this standard the annexes A and B are normative. The annexes C, D, E, F, G and H are informative.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

## **Introduction**

This European Standard describes a suspension test method for establishing whether a chemical disinfectant has or does not have a sporicidal activity in the fields described in clause 1.

The laboratory test closely simulates practical conditions of application. Chosen conditions (contact time, temperature, in suspension, etc.) reflect parameters which are found in practical situations including conditions which can influence the action of disinfectants. Each utilization concentration found from this test corresponds to defined experimental conditions.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types.

However for some applications the recommendations of use of a product can differ and therefore additional test conditions need to be used.

## 1 Scope

This European Standard specifies a test method (phase 2/step 1) (see annex H) and the minimum requirements for sporicidal activity of chemical disinfectant products that form a homogeneous, physically stable preparation in hard water and that are used in food, industrial, domestic and institutional areas, excluding areas and situations where disinfection is medically indicated and excluding products used on living tissues except those for hand hygiene in the above considered areas.

This European Standard applies at least to the following :

a) processing, distribution and retailing of :

1) food of animal origin :

- milk and milk products ;
- meat and meat products ;
- fish, seafood, and related products ;
- eggs and egg products ;
- animal feeds ;
- etc. ;

2) food of vegetable origin :

- beverages ;
- fruits, vegetables and derivatives (including sugar, distillery, etc.) ;
- flour, milling and baking ;
- animal feeds ;
- etc. ;

b) institutional and domestic areas :

- catering establishments ;
- public areas ;
- public transports ;
- schools ;
- nurseries ;
- shops ;
- sports rooms ;
- waste containers (bins, etc.) ;
- hotels ;

- dwellings ;
  - clinically non sensitive areas of hospitals ;
  - offices ;
  - etc. ;
- c) other industrial areas :
- packaging material ;
  - biotechnology (yeast, proteins, enzymes, etc.) ;
  - pharmaceutical ;
  - cosmetics and toiletries ;
  - textiles ;
  - space industry, computer industry ;
  - etc.

Using this European Standard, it is not possible to determine the sporicidal activity of undiluted product as some dilution is always produced by adding the inoculum and interfering substance. Products can only be tested at a concentration of 80 % or less.

NOTE The method described is intended to determine the activity of commercial formulations or active substances on spores in the conditions in which they are used.

## 2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN 1040, *Chemical disinfectants and antiseptics – Basic bactericidal activity – Test method and requirements (phase 1)*.

## 3 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply.

### 3.1

#### **product (for chemical disinfection and/or antiseptics)**

chemical agent or formulation used as a chemical disinfectant or antiseptic [EN 1040]

### 3.2

#### **sporicide** (in this standard)

product which kills spores of the genus *Bacillus*

NOTE The adjective derived from « sporicide » is « sporicidal ».

**3.3****sporicidal activity** (in this standard)

capability of the product to produce at least a reduction of  $10^3$  in the number of bacterial spores belonging to reference strain of *Bacillus subtilis* under conditions defined by this European Standard

**3.4****clean conditions**

conditions representative of surfaces which have received a satisfactory cleaning programme and/or are known to contain minimal levels of organic and/or inorganic materials

**4 Requirements**

The product, when diluted in hard water and tested in accordance with clause 5 under simulated clean conditions (0,3 g/l bovine albumin see 3.4) according to its practical applications and under the required test conditions (20 °C, 60 min, 1 selected reference strain), shall demonstrate at least a  $10^3$  reduction in viable counts.

The sporicidal activity shall be evaluated using the test organism *Bacillus subtilis*.

The determined sporicidal concentration of the test product is suggested as being suitable for practical situations of use.

Where appropriate, additional specific sporicidal activity shall be determined under other conditions of time, temperature and additional strains (see 5.2.1 and 5.5.1) in accordance with 5.5.1 in order to take into account intended specific use conditions.

NOTE For these additional conditions, the concentration defined as a result can be lower than the one obtained under the initial test conditions of 20 °C, 60 min, 1 selected reference strain.

**5 Test method****5.1 Principle**

**5.1.1** A test suspension of bacterial spores in a solution of interfering substance, simulating clean conditions, is added to a prepared sample of the product under test diluted in hard water. The mixture is maintained at  $20\text{ °C} \pm 1\text{ °C}$  for  $60\text{ min} \pm 10\text{ s}$  (required test conditions).

At this contact time, an aliquot is taken ; the sporicidal action in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The number of surviving bacterial spores in each sample are determined and the reduction in viable counts is calculated.

**5.1.2** The test is performed using spores of *Bacillus subtilis*. Additional and optional exposure times, temperatures and strains are specified.

## 5.2 Materials and reagents

### 5.2.1 Test organisms

The sporicidal activity shall be evaluated by using spores of the following strain :

— *Bacillus subtilis* ATCC 6633 <sup>1)</sup>.

If required for specific applications, additional strains may be chosen from, for example :

— *Bacillus cereus* ATCC 12826 <sup>1)</sup> ;

— *Clostridium sporogenes* CIP 7939 <sup>1)</sup>.

NOTE 1 See annex G for corresponding strain numbers in some other culture collections.

NOTE 2 See annex C for particular culture and handling conditions for *Clostridium sporogenes*.

If additional strains are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere) and noted in the test report.

If the additional strains selected do not correspond to the specified strains, their suitability for supplying inocula of sufficient concentration shall be verified. If the additional strains tested are not classified at a reference centre their identification characteristics shall be stated. In addition, they shall be held by the testing laboratory or national culture under a reference for 5 years.

### 5.2.2 Culture media and reagents

#### 5.2.2.1 General

The reagents shall be of analytical grade and/or appropriate for microbiological purposes.

NOTE To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

#### 5.2.2.2 Water

The water shall be free from substances that are toxic or inhibiting to the bacterial spores or to the bacteria. It shall be freshly glass distilled water and not demineralized water.

Sterilize in the autoclave (see 5.3.1).

NOTE 1 If the water is sterilized during the sterilization of the reagents, this is not necessary.

NOTE 2 If distilled water of adequate quality is not available, water for injectable preparation\_(European Pharmacopoeia) can be used.

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<sup>1)</sup> ATCC 6633 and ATCC 12826 are the collection numbers of strains supplied by the American Type Culture Collections. CIP 7939 is the collection number of spores supplied by the Collection de l'Institut Pasteur. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the product named. Corresponding strains supplied by other culture collections may be used if they can be shown to lead to the same results.

### 5.2.2.3 Glucose Yeast Extract Agar (GYA)

For counting of viable *Bacillus* spores :

|                                                                            |            |
|----------------------------------------------------------------------------|------------|
| Amino-acids, without vitamins, obtained by acid hydrolysis of casein ..... | 1,0 g      |
| Soluble starch.....                                                        | 1,0 g      |
| Glucose .....                                                              | 2,5 g      |
| Yeast extract .....                                                        | 5,0 g      |
| FeSO <sub>4</sub> .....                                                    | 0,1 g      |
| MnSO <sub>4</sub> · H <sub>2</sub> O .....                                 | 0,000 1 g  |
| Agar .....                                                                 | 15,0 g     |
| Water (see 5.2.2.2) .....                                                  | 1 000,0 ml |

Sterilize in the autoclave (see 5.3.1). After sterilization the pH of the medium shall be equivalent to  $6,8 \pm 0,2$  when measured at 20 °C.

### 5.2.2.4 Neutralizer

The neutralizer shall be validated for the product under test in accordance with annex D. The neutralizer shall be sterile.

NOTE Information on neutralizers that have been found to be suitable for some categories of products is given in annex D.

### 5.2.2.5 Rinsing liquid (for membrane filtration)

The liquid shall be sterile, compatible with the filter membrane and capable of filtration through the filter membrane under the test conditions described in annex B.

NOTE Information on rinsing liquids that have been found to be suitable for some categories of products is given in annex E.

### 5.2.2.6 Hard water for dilution of products

Hard water for dilution of products shall be prepared as follows :

- Solution A : Dissolve 19,84 g anhydrous magnesium chloride (MgCl<sub>2</sub>) or an equivalent of hydrated magnesium chloride and 46,24 g anhydrous calcium chloride (CaCl<sub>2</sub>) or an equivalent of hydrated calcium chloride in water (see 5.2.2.2) and dilute to 1000 ml.

Sterilize in the autoclave (see 5.3.1). Store the solution at 2 °C - 8 °C for no longer than one month.

- Solution B : Dissolve 35,02 g sodium bicarbonate (NaHCO<sub>3</sub>) in water (see 5.2.2.2) and dilute to 1 000 ml. Sterilize by membrane filtration (see 5.3.2.7). Store the solution at 2 °C - 8 °C for no longer than one week.

Hard Water :

For the preparation of 1 litre, place at least 600 ml water (see 5.2.2.2) in a 1000 ml volumetric flask (see 5.3.2.12) and add 6,0 ml of solution A, then 8,0 ml of solution B.

Mix and dilute to 1 000 ml with water (see 5.2.2.2).

The pH of the hard water shall be  $7,0 \pm 0,2$ .

If necessary adjust the pH by using a solution of approximately 40 g/l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid (HCl).

The hard water shall be freshly prepared under aseptic conditions and used within 12 hours.

NOTE When preparing the product test solutions (see 5.4.2) the addition of the product to this hard water produces a different final water hardness in each test tube.

In any case the final hardness is lower than 300 mg/kg of calcium carbonate ( $\text{CaCO}_3$ ) in the test tube.

### 5.2.2.7 Interfering substance

#### 5.2.2.7.1 General

The interfering substance shall be sterile and prepared at 10 times its final concentration in the test.

#### 5.2.2.7.2 Bovine albumin solution

Bovine albumin solution for the test conditions shall be prepared as follows :

- dissolve 0,30 g of bovine albumin (Cohn fraction V for Dubos medium) in 100 ml of water (see 5.2.2.2) ;
- sterilize by membrane filtration.

The final concentration of the bovine albumin in the test procedure (see 5.5.2) is 0,3 g/l.

## 5.3 Apparatus and glassware

### 5.3.1 General

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those which are supplied sterile, by one of the following methods :

- a) in the autoclave (see 5.3.2.1) by maintaining it at  $(121^{+3}_0)$  °C for a minimum holding time of 15 min ;
- b) in the dry heat sterilizer (see 5.3.2.1) by maintaining it at 180 °C for a minimum holding time of 30 min, at 170 °C for a minimum holding time of 1 h, or at 160 °C for a minimum holding time of 2 h.

### 5.3.2 Usual microbiological laboratory equipment <sup>2)</sup> and, in particular, the following :

#### 5.3.2.1 Apparatus for sterilization

- a) for moist heat sterilization, an autoclave capable of being maintained at  $(121^{+3}_0)$  °C for a minimum holding time of 15 min ;
- b) for dry heat sterilization, a hot air oven capable of being maintained at 180 °C for a minimum holding time of 30 min, at 170 °C for a minimum holding time of 1 h, or at 160 °C a minimum holding time of 2 h.

**5.3.2.2 Water baths**, capable of being controlled at 20 °C  $\pm$  1 °C, 45 °C  $\pm$  1 °C and at additional test temperatures  $\pm$  1 °C (see 5.5.1).

**5.3.2.3 Incubator**, capable of being controlled at 30 °C  $\pm$  1 °C.

**5.3.2.4 pH-meter**, having an accuracy of calibration of  $\pm$  0,1 pH units at 25 °C.

**5.3.2.5 Stopwatch**

**5.3.2.6 Vortex mixer (electromechanical agitator, i.e. Vortex® mixer <sup>3)</sup>)**

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<sup>2)</sup> Disposable equipment is an acceptable alternative to reusable glassware.