

# **SLOVENSKI STANDARD**

## **SIST EN 1040:2006**

**01-junij-2006**

**Nadomešča:**  
**SIST EN 1040:2001**

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**Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje osnovnega baktericidnega delovanja kemičnih razkužil in antiseptikov - Preskusna metoda in zahteve (faza 1)**

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics - Test method and requirements (phase 1)

**ITeh STANDARD PREVIEW**

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Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der bakteriziden Wirkung (Basistest) chemischer Desinfektionsmittel und Antiseptika - Prüfverfahren und Anforderungen (Phase 1)

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Désinfectants et antiseptiques chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité de base bactéricide antiseptiques et des désinfectants chimiques - Méthode d'essai et prescriptions (phase 1)

**Ta slovenski standard je istoveten z: EN 1040:2005**

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71.100.35	Kemikalije za dezinfekcijo v industriji in doma	Chemicals for industrial and domestic disinfection purposes

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**en**

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EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

**EN 1040**

December 2005

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Supersedes EN 1040:1997

English Version

**Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics - Test method and requirements (phase 1)**

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide de base des antiseptiques et des désinfectants chimiques - Méthode d'essai et prescriptions (phase 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der bakteriziden Wirkung (Basistest) chemischer Desinfektionsmittel und Antiseptika - Prüfverfahren und Anforderungen (Phase 1)

This European Standard was approved by CEN on 28 July 2005.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

<b>Contents</b>	<b>Page</b>
<b>FOREWORD .....</b>	<b>3</b>
<b>INTRODUCTION .....</b>	<b>4</b>
<b>1 SCOPE .....</b>	<b>5</b>
<b>2 NORMATIVE REFERENCES .....</b>	<b>5</b>
<b>3 TERMS AND DEFINITIONS .....</b>	<b>5</b>
<b>4 REQUIREMENTS .....</b>	<b>6</b>
<b>5 TEST METHOD .....</b>	<b>6</b>
5.1 Principle .....	6
5.2 Materials and reagents .....	6
5.3 Apparatus and glassware .....	8
5.4 Preparation of test organism suspensions and product test solutions .....	9
5.5 Procedure for assessing the bactericidal activity of the product .....	11
5.6 Experimental data and calculation .....	16
5.7 Verification of methodology .....	20
5.8 Expression of results and precision .....	21
5.9 Interpretation of results – conclusion .....	21
5.10 Test report .....	22
<b>ANNEX A (INFORMATIVE) REFERENCED STRAINS IN NATIONAL COLLECTIONS .....</b>	<b>24</b>
<b>ANNEX B (INFORMATIVE) SUITABLE NEUTRALIZERS AND RINSING LIQUIDS .....</b>	<b>25</b>
<b>ANNEX C (INFORMATIVE) GRAPHICAL REPRESENTATION OF TEST PROCEDURES .....</b>	<b>27</b>
<b>ANNEX D (INFORMATIVE) EXAMPLE OF A TYPICAL TEST REPORT .....</b>	<b>31</b>
<b>ANNEX E (INFORMATIVE) PRECISION OF THE TEST RESULT .....</b>	<b>34</b>
<b>ANNEX F (INFORMATIVE) INFORMATION ON THE APPLICATION AND INTERPRETATION OF EUROPEAN STANDARD ON CHEMICAL DISINFECTANTS AND ANTISEPTICS .....</b>	<b>38</b>
<b>BIBLIOGRAPHY .....</b>	<b>40</b>

## Foreword

This European Standard (EN 1040:2005) 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 June 2006, and conflicting national standards shall be withdrawn at the latest by June 2006.

This European Standard supersedes EN 1040:1997.

It was revised to correct obvious errors and ambiguities, to harmonize the structure and wording with other quantitative suspension tests of CEN TC 216 existing or in preparation and to improve the readability and with that the understandability of the standard.

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

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## Introduction

This European Standard specifies a suspension test for establishing whether a chemical disinfectant or antiseptic does or does not have a *basic* bactericidal activity in the fields described in the scope. The acceptability of a product for a defined purpose cannot be determined from this test method. Therefore products are subjected to further testing by relevant tests specified in European Standards in order to evaluate their activity under conditions appropriate to their intended use. These European Standards have been or will be developed by CEN/TC 216.

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## 1 Scope

This European Standard specifies a test method and the minimum requirements for basic bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with water. Products can only be tested at a concentration of 80 % or less as some dilution is always produced by adding the test organisms and water.

This European Standard applies to active substances (antibacterial biocides) and to formulations under development that are planned to be used in food, industrial, domestic and institutional, medical and veterinary areas. It applies also to the evaluation of bactericidal activity of chemical antiseptics and disinfectants when appropriate standards are not available.

NOTE 1 This European Standard does not evaluate the activity of a product for an intended use.

NOTE 2 This method corresponds to a phase 1 test (Annex F).

## 2 Normative references

The following referenced documents are indispensable for the application of this European Standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12353, *Chemical disinfectants and antiseptics – Preservation of microbial strains used for the determination of bactericidal and fungicidal activity*

## 3 Terms and definitions

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

### 3.1

#### **product**

chemical agent or formulation used as chemical disinfectant or antiseptic

### 3.2

#### **bactericide**

product that kills vegetative bacteria under defined conditions

NOTE The adjective derived from “bactericide” is “bactericidal”.

### 3.3

#### **bactericidal activity**

capability of a product to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions

### 3.4

#### **bacteriostatic activity**

capability of a product to inhibit the growth of bacteria under defined conditions

## EN 1040:2005 (E)

## 4 Requirements

The product shall demonstrate at least a 5 decimal log (lg) reduction when tested in accordance with Clause 5.

The bactericidal activity shall be evaluated using at least the following obligatory experimental test conditions: two test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*), 20 °C, 5 min.

Where indicated, bactericidal activity could be determined applying additional contact times, temperatures and test organisms in accordance with 5.2.1 and 5.5.1.1.

NOTE 1 For these additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions.

NOTE 2 At the concentration defined as a result, it is not necessary to demonstrate a 5 lg reduction with the obligatory test conditions.

## 5 Test method

### 5.1 Principle

5.1.1 A sample of the product as delivered (highest test concentration = 80 %) and/or diluted with water is added to a test suspension of bacteria. The mixture is maintained at  $(20 \pm 1) ^\circ\text{C}$  for  $5 \text{ min} \pm 10 \text{ s}$  (obligatory test conditions). At the end of this contact time, an aliquot is taken, the bactericidal and/or the bacteriostatic activity 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 numbers of surviving bacteria in each sample are determined and the reduction is calculated.

5.1.2 The test is performed using *Pseudomonas aeruginosa* and *Staphylococcus aureus* as test organisms (obligatory test conditions).

5.1.3 Additional and optional contact times and temperatures are specified. Additional test organisms can be used.

### 5.2 Materials and reagents

#### 5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following strains as test organisms:<sup>1)</sup>

- *Pseudomonas aeruginosa* ATCC 15442;
- *Staphylococcus aureus* ATCC 6538.

NOTE See Annex A for strain references in some other culture collections.

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<sup>1)</sup> The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collection (ATCC). This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named.



The required incubation temperature for these test organisms is  $(36 \pm 1) ^\circ\text{C}$  or  $(37 \pm 1) ^\circ\text{C}$  (**5.3.2.3**). The same temperature (either  $36 ^\circ\text{C}$  or  $37 ^\circ\text{C}$ ) shall be used for all incubations performed during a test and its control and validation.

If additional test organisms are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere, media) noted in the test report. If the additional test organisms selected do not correspond to the specified strains, their suitability for supplying the required inocula shall be verified. If these additional test organisms 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 collection under a reference for five years.

## 5.2.2 Culture media and reagents

### 5.2.2.1 General

All weights of chemical substances given in this European Standard refer to the anhydrous salts. Hydrated forms may be used as an alternative, but the weights required shall be adjusted to allow for consequent molecular weight differences.

The reagents shall be of analytical grade and/or appropriate for microbiological purposes. They shall be free from substances that are toxic or inhibitory to the test organisms.

NOTE 1 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.

NOTE 2 For each culture medium and reagent, a limitation for use should be fixed.

### 5.2.2.2 Water

The water shall be freshly glass-distilled water and not demineralized water.

Sterilize in the autoclave [**5.3.2.1a**].

NOTE 1 Sterilization is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilized.

NOTE 2 If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1]) can be used.

### 5.2.2.3 Tryptone Soya Agar (TSA)

Tryptone soya agar, consisting of:

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
Sodium chloride (NaCl)	5,0 g
Agar	15,0 g
Water ( <b>5.2.2.2</b> )	to 1 000,0 ml

Sterilize in the autoclave [**5.3.2.1a**]. After sterilization the pH of the medium shall be equivalent to  $(7,2 \pm 0,2)$  when measured at  $(20 \pm 1) ^\circ\text{C}$ .

NOTE In case of encountering problems with neutralization (**5.5.1.2** and **5.5.1.3**) it may be necessary to add neutralizer to the TSA. **Annex B** gives guidance on the neutralizers that may be used.

**EN 1040:2005 (E)****5.2.2.4 Diluent**

Tryptone sodium chloride solution, consisting of:

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave [5.3.2.1a)]. After sterilization, the pH of the diluent shall be equivalent to  $(7,0 \pm 0,2)$  when measured at  $(20 \pm 1) ^\circ\text{C}$ .

**5.2.2.5 Neutralizer**

The neutralizer shall be validated for the product being tested in accordance with 5.5.1.2, 5.5.1.3 and 5.5.2. It shall be sterile.

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

**5.2.2.6 Rinsing liquid (for membrane filtration)**

The rinsing liquid shall be validated for the product being tested in accordance with 5.5.1.2, 5.5.1.3 and 5.5.3. It shall be sterile, compatible with the filter membrane and capable of filtration through the filter membrane under the test conditions described in 5.5.3.

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

**5.3 Apparatus and glassware**

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**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) by moist heat, in the autoclave [5.3.2.1a)];
- b) by dry heat, in the hot air oven [5.3.2.1b)].

**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  $(121^{+3}_0) ^\circ\text{C}$  for a minimum holding time of 15 min;
- b) for dry heat sterilization, a hot air oven capable of being maintained at  $(180^{+5}_0) ^\circ\text{C}$  for a minimum holding time of 30 min, at  $(170^{+5}_0) ^\circ\text{C}$  for a minimum holding time of 1 h or at  $(160^{+5}_0) ^\circ\text{C}$  for a minimum holding time of 2 h.

**5.3.2.2 Water baths**, capable of being controlled at  $(20 \pm 1) ^\circ\text{C}$ , at  $(45 \pm 1) ^\circ\text{C}$  (to maintain melted TSA in case of pour plate technique) and at additional test temperatures  $\pm 1 ^\circ\text{C}$  (5.5.1).

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

**5.3.2.3 Incubator**, capable of being controlled either at  $(36 \pm 1) ^\circ\text{C}$  or  $(37 \pm 1) ^\circ\text{C}$  (**5.2.1**).

**5.3.2.4 pH-meter**, having an inaccuracy of calibration of no more than  $\pm 0,1$  pH units at  $(20 \pm 1) ^\circ\text{C}$ .

NOTE (5.2.2.3). A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar media (5.2.2.3).

**5.3.2.5 Stopwatch.**

**5.3.2.6 Shakers**

a) Electromechanical agitator, e.g. Vortex<sup>®</sup> mixer <sup>3)</sup>.

b) Mechanical shaker.

**5.3.2.7 Membrane filtration apparatus**, constructed of a material compatible with the substances to be filtered.

The apparatus shall have a filter holder of at least 50 ml volume. It shall be suitable for use with filters of diameter 47 mm to 50 mm and 0,45  $\mu\text{m}$  pore size for the membrane filtration method (**5.5.3**).

The vacuum source used shall give an even filtration flow rate. In order to obtain a uniform distribution of the micro-organisms over the membrane and to prevent overlong filtration, the device shall be set so as to obtain the filtration of 100 ml of rinsing liquid in 20 s to 40 s.

**5.3.2.8 Refrigerator**, capable of being controlled at  $2 ^\circ\text{C}$  to  $8 ^\circ\text{C}$ .

**5.3.2.9 Graduated pipettes**, of nominal capacities 10 ml, 1 ml and 0,1 ml, or calibrated automatic pipettes.

**5.3.2.10 Petri dishes (plates)**, of size 90 mm to 100 mm.

**5.3.2.11 Glass beads**, 3 mm to 4 mm in diameter.

**5.3.2.12 Volumetric flasks.**

## 5.4 Preparation of test organism suspensions and product test solutions

### 5.4.1 Test organism suspensions (test and validation suspension)

#### 5.4.1.1 General

For each test organism, two different suspensions have to be prepared: the "test suspension" to perform the test and the "validation suspension" to perform the controls and method validation.

#### 5.4.1.2 Preservation and stock cultures of test organisms

The test organisms and their stock cultures shall be prepared and kept in accordance with EN 12353.

#### 5.4.1.3 Working culture of test organisms

In order to prepare the working culture of the test organisms (**5.2.1**), prepare a subculture from the stock culture (**5.4.1.2**) by streaking onto TSA (**5.2.2.3**) slopes or plates and incubate (**5.3.2.3**). After 18 h to 24 h prepare a second subculture from the first subculture in the same way and incubate for 18 h to 24 h. From this

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<sup>3)</sup> Vortex<sup>®</sup> is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

**EN 1040:2005 (E)**

second subculture, a third subculture may be produced in the same way. The second and (if produced) third subcultures are the working cultures.

If it is not possible to prepare the second subculture on a particular day, a 48 h subculture may be used for subsequent subculturing, provided that the subculture has been kept in the incubator (5.3.2.3) during the 48 h period.

Never produce and use a fourth subculture.

For additional test organisms, any departure from this method of culturing the test organisms or of preparing the suspensions shall be noted, giving the reasons in the test report.

**5.4.1.4 Test suspension ("N")**

- a) Take 10 ml of diluent (5.2.2.4) and place in a 100 ml flask with 5 g of glass beads (5.3.2.11). Take the working culture (5.4.1.3) and transfer loopfuls of the cells into the diluent (5.2.2.4). The cells should be suspended in the diluent by rubbing the loop against the wet wall of the flask to dislodge the cells before immersing in the diluent. Shake the flask for 3 min using a mechanical shaker [(5.3.2.6b)]. Aspirate the suspension from the glass beads and transfer to another tube.
- b) Adjust the number of cells in the suspension to  $1,5 \times 10^8$  cfu/ml<sup>4)</sup> to  $5 \times 10^8$  cfu/ml using diluent (5.2.2.4), estimating the number of cfu by any suitable means. Maintain this test suspension in the water bath at the test temperature  $\theta$  [5.5.1.1a)] and use within 2 h.

NOTE The use of a spectrophotometer for adjusting the number of cells is highly recommended (about 620 nm wavelength - cuvette 10 mm path length). Each laboratory should therefore produce calibration data for each test organism knowing that suitable values of optical density are generally found between 0,150 and 0,460. A colorimeter is a suitable alternative.

- c) For counting, prepare  $10^{-6}$  and  $10^{-7}$  dilutions of the test suspension using diluent (5.2.2.4). Mix [(5.3.2.6a)]. Take a sample of 1,0 ml of each dilution in duplicate and inoculate using the pour plate or the spread plate technique.
  - 1) When using the pour plate technique, transfer each 1,0 ml sample into separate Petri dishes and add 15 ml to 20 ml melted TSA (5.2.2.3), cooled to  $(45 \pm 1) ^\circ\text{C}$ .
  - 2) When using the spread plate technique, spread each 1,0 ml sample – divided into portions of approximately equal size – on an appropriate number (at least two) of surface dried plates containing TSA (5.2.2.3).

For incubation and counting see 5.4.1.6.

**5.4.1.5 Validation suspension ("N<sub>v</sub>")**

- a) To prepare the validation suspension, dilute the test suspension (5.4.1.4) with the diluent (5.2.2.4) to obtain  $3,0 \times 10^2$  cfu/ml to  $1,6 \times 10^3$  cfu/ml [about one fourth (1+3) of the  $10^{-5}$  dilution].
- b) For counting prepare a  $10^{-1}$  dilution with diluent (5.2.2.4). Mix [(5.3.2.6a)]. Take a sample of 1,0 ml in duplicate and inoculate using the pour plate or the spread plate technique [5.4.1.4 c)].

For incubation and counting see 5.4.1.6.

**5.4.1.6 Incubation and counting of the test and the validation suspensions**

- a) Incubate (5.3.2.3) the plates for 20 h to 24 h. Discard any plates that are not countable for any reason. Count the plates and determine the number of cfu. Incubate the plates for a further 20 h to 24 h. Do not

<sup>4)</sup> cfu/ml = colony forming unit(s) per millilitre.

recount plates that no longer show well-separated colonies. Recount the remaining plates. If the number has increased, use only the higher number for further evaluation.

- b) Note for each plate the exact number of colonies but record  $> 330$  for any counts higher than 330 and determine the  $V_c$  values according to 5.6.2.2.
- c) Calculate the numbers of cfu/ml in the test suspension "N" and in the validation suspension "Nv" using the methods given in 5.6.2.3 and 5.6.2.5. Verify according to 5.7.

#### 5.4.2 Product test solutions

The concentration of a product test solution shall be 1,25 times the desired test concentration because it is diluted to 80 % during the test and the method validation (5.5.2 or 5.5.3). Product test solutions shall be prepared in water (5.2.2.2) at minimum three different concentrations to include one concentration in the active range and one concentration in the non-active range (5.8.2). The product as received may be used as one of the product test solutions, in this case the highest tested concentration is 80 %.

For solid products, dissolve the product as received by weighing at least  $1,0 \text{ g} \pm 10 \text{ mg}$  of the product in a volumetric flask and filling up with water (5.2.2.2). Subsequent dilutions (lower concentrations) shall be prepared in volumetric flasks (5.3.2.12) on a volume/volume basis in water (5.2.2.2).

For liquid products, dilutions of the product shall be prepared with water (5.2.2.2) on a volume/volume basis using volumetric flasks (5.3.2.12).

The product test solutions shall be prepared freshly and used in the test within 2 h. They shall give a physically homogeneous preparation that is stable during the whole procedure. If during the procedure a visible inhomogeneity appears due to the formation of a precipitate or flocculant, it shall be recorded in the test report.

NOTE Counting micro-organisms embedded in a precipitate or flocculant is difficult and unreliable.

The concentration of the product stated in the test report shall be the desired test concentration. Record the test concentration in terms of mass per volume or volume per volume and details of the product sample as received.

### 5.5 Procedure for assessing the bactericidal activity of the product

#### 5.5.1 General

##### 5.5.1.1 Experimental conditions (obligatory and additional)

Besides the obligatory temperature, contact time and test organisms additional experimental conditions may be selected (Clause 4), as follows:

- a) temperature  $\theta$  (in  $^{\circ}\text{C}$ ):
  - the obligatory temperature to be tested is  $\theta = 20^{\circ}\text{C}$ ;
  - the additional temperatures may be chosen from  $4^{\circ}\text{C}$ ,  $10^{\circ}\text{C}$  or  $40^{\circ}\text{C}$ ;
  - the allowed deviation for each chosen temperature is  $\pm 1^{\circ}\text{C}$ .
- b) contact time  $t$  (in min):
  - the obligatory contact time to be tested is  $t = 5 \text{ min}$ ;
  - the additional contact times may be chosen from 1 min, 15 min, 30 min or 60 min;
  - the allowed deviation for each chosen contact time is  $\pm 10 \text{ s}$ , except for 1 min, for which it is  $\pm 5 \text{ s}$ .