



SLOVENSKI STANDARD

SIST EN 1104:2005

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Paper and board intended to come into contact with foodstuffs - Determination of the transfer of antimicrobial constituents

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Papier und Pappe vorgesehen für den Kontakt mit Lebensmitteln - Bestimmung des Übergangs antimikrobieller Bestandteile

SIST EN 1104:2005

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Papier et carton destinés à entrer en contact avec des denrées alimentaires - Détermination du transfert des constituants antimicrobiens

Ta slovenski standard je istoveten z: EN 1104:2005

ICS:

67.250	Materiali in predmeti v stiku z živili	Materials and articles in contact with foodstuffs
85.060	Papir, karton in lepenka	Paper and board

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English Version

**Paper and board intended to come into contact with foodstuffs -
Determination of the transfer of antimicrobial constituents**

Papier et carton destinés à entrer en contact avec des
denrées alimentaires - Détermination du transfert des
constituants antimicrobiens

Papier und Pappe vorgesehen für den Kontakt mit
Lebensmitteln - Bestimmung des Übergangs
antimikrobieller Bestandteile

This European Standard was approved by CEN on 27 June 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.

CEN members are the national standards bodies of 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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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This European Standard (EN 1104:2005) has been prepared by Technical Committee CEN/TC 172 “Pulp, paper and board”, the secretariat of which is held by DIN.

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 February 2006, and conflicting national standards shall be withdrawn at the latest by February 2006.

This European Standard supersedes EN 1104:1995.

With regard to EN 1104:1995 the following changes have been made:

- a) description of the preparation of some reagents were partly modified;
- b) more detailed description of the procedure was formulated;
- c) editorial updating.

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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EN 1104:2005 (E)

1 Scope

This European Standard specifies a method for the determination of transfer of antimicrobial constituents from paper and board materials and articles intended for food contact.

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 ISO 186, *Paper and board — Sampling to determine average quality (ISO 186:2002)*

3 Terms and definitions

For the purposes of this European Standard, the following term and definition applies.

3.1

inhibition zone

zone formed around the test pieces, placed on nutrient medium, which has been seeded with a pre-selected test organism that releases water-soluble antimicrobial constituents.

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4 Principle

A prepared nutrient medium is mixed with an appropriate inoculum and poured into Petri dishes. The test pieces are placed on the semi solid nutrient medium and then incubated. When incubation is terminated, the existence of an inhibition zone is an indicator of the release of antimicrobial constituents.

The test is performed with a bacterium, *Bacillus subtilis*, and with a fungus, *Aspergillus niger*.

NOTE The result is based on a visual inspection.

5 Apparatus

5.1 Punch iron,

$d = 10$ mm to 15 mm, sterilizable.

5.2 Pressing device,

suitable for pressing the test pieces on the agar plate (e.g. Drygalski spatula).

5.3 Zone reading device,

to measure the diameter of inhibition.

NOTE Measuring the diameter of the inhibition zone is not compulsory.

5.4 Ordinary microbiological laboratory apparatus

6 Reagents

6.1 Water,

freshly distilled or water purified by ion exchange and freshly boiled (deionised water).

6.2 Non ionic wetting agent,

for example polyoxyethylenesorbitane monooleate.

6.3 Nutrient medium for *Bacillus subtilis*

A typical formulation of the nutrient medium is:

— beef extract	3,0 g;
— tryptone (peptone of casein)	5,0 g;
— sodium chloride, pure	5,0 g;
— agar-agar	12,0 g
— water	1000,0 ml

NOTE The addition of 10 mg/l $MnSO_4$ to the nutrient medium for *Bacillus subtilis* (6.7.1) will support the formation of spores.

Prepare the nutrient medium as follows: <https://standards.iteh.ai/catalog/standards/sist/7a5b7475-da4c-4b40-8d41-59662bec7/sist-en-1104-2005>

Dissolve the components, or a ready-made medium of a comparable composition, in water by boiling.

The pH of the ready prepared nutrient medium shall be $(7,2 \pm 0,2)$ referred at a temperature of 45 °C.

Adjust the pH to $(7,2 \pm 0,2)$ as required either with NaOH approx. 0,01 M or HCl approx. 0,01 M.

Separate the nutrient medium into two parts.

Dispense one part in 300,0 ml portions into nutrient medium flasks or 600,0 ml flasks e.g. Roux flasks and stopper them with caps, e.g. Kapsenberg caps.

Use the other part for the preparation of the working culture media into test tubes.

Dispense 10,0 ml portions into 15 to 20 test tubes and seal them with stoppers, e.g. cellulose stoppers.

Sterilise flasks and test tubes for 15 min at (121 ± 1) °C. After sterilisation position the test tubes immediately in such a way that the nutrient medium solidifies with a sloping surface.

Store them at 4 °C to 8 °C not longer than 14 d.

Cool the nutrient medium flasks to approx. 45 °C for the preparation of the inoculating suspension of *Bacillus subtilis* (6.7) or allow to solidify.

Cool the flasks to solid.

EN 1104:2005 (E)**6.4 Sabouraud modified mould nutrient medium for *Aspergillus niger***

A typical formulation of the Sabouraud modified mould nutrient medium is:

- tryptone (peptone of casein) 5,0 g
- peptone (peptone of meat) 5,0 g;
- D (+) glucose $C_6H_{12}O_6 \cdot H_2O$ 10,0 g;
- maltose $C_{12}H_{22}O_{11} \cdot H_2O$ 10,0 g;
- agar-agar 10,0 g to 15,0 g;
- water 1000,0 ml

Prepare the Sabouraud modified mould nutrient medium as follows:

Dissolve the components, or a ready-made medium of a comparable composition, by boiling.

The pH of the ready prepared nutrient medium shall be $(5,4 \pm 0,1)$ referred at a temperature of 45 °C.

Adjust the pH to $(5,4 \pm 0,1)$ as required either with NaOH approx. 0,01 M or HCl approx. 0,01 M.

Proceed as described in 6.3 dispensing the medium into nutrient medium flasks or flasks e.g. Roux flasks and test tubes and the sterilising and cooling procedures.

6.5 Nutrient medium for inhibition test with *Bacillus subtilis*

The composition of the nutrient medium for inhibition test with *Bacillus subtilis* shall be as follows:

- tryptone (peptone of casein) 3,45 g,
- peptone (peptone of meat) 3,45 g;
- sodium chloride, pure 5,1 g;
- agar-agar 13,0 g;
- water 1000,0 ml.

Prepare the nutrient medium as follows:

Dissolve the components, or a ready-made medium of a comparable composition, in water by boiling.

The pH of the ready made nutrient medium shall be $(6,0 \pm 0,1)$ referred at a temperature of 45 °C. Adjust the pH to $(6,0 \pm 0,1)$ as required either with NaOH approx. 0,01 M or HCl approx. 0,01 M.

Dispense portions of nutrient medium flasks or test tubes and stopper them with caps. e.g. Kapsenberg caps and sterilise for 15 min at (121 ± 1) °C.

Cool the flasks to below 60 °C for the preparation of the inoculation medium (8.2.2) or allow to solidify.

6.6 Salt peptone solution

The composition of the salt peptone solution shall be as follows:

- peptone (peptone of meat) 1,0 g;
- sodium chloride, pure 8,5 g;
- water 1000,0 ml.

Prepare the salt peptone solution as follows:

Dissolve the components in water of pH between 6 and 7. Dispense equal volumes into three flasks, stopper with caps, e.g. Kaspensberg caps and sterilise for 15 min at $(121 \pm 1) ^\circ\text{C}$.

The solution shall be used within 8 d if stored at room temperature and within 14 d when stored at $4 ^\circ\text{C}$ to $8 ^\circ\text{C}$.

6.7 Test micro-organisms

6.7.1 General

The following are used:

Bacillus subtilis DSM 347 (ATCC 6633) and *Aspergillus niger* DSM 1957 (ATCC 6275) or other corresponding strains.

As different strains may also have different sensitivity, a comparative examination would have to be performed when using other strains than ATCC 6633 and ATCC 6275.

Working cultures of *Bacillus subtilis* are obtained by inoculating onto the test tubes (6.3) and incubating for 7 d at $30 ^\circ\text{C}$. After incubation the test tubes are stored at $4 ^\circ\text{C}$ to $8 ^\circ\text{C}$.

Working cultures of *Aspergillus niger* are obtained by inoculating onto the test tubes (6.4) and incubating for 5 d at $25 ^\circ\text{C}$. After incubating the test tubes are stored at $4 ^\circ\text{C}$ to $8 ^\circ\text{C}$.

6.7.2 Preparation of inoculating spore suspension of *Bacillus subtilis*

Transfer aliquots of about 15,0 ml of the liquefied nutrient medium (6.3) cooled to approximately $45 ^\circ\text{C}$ to 10 sterile Petri dishes ($d = 90$ mm) and allow to solidify.

The nutrient medium in flasks (6.3) is ready for inoculation.

Wash off the colonies of ten test tubes with the working culture of *Bacillus subtilis* (6.7.1) with 2,0 ml to 3,0 ml sterile salt, peptone solution (6.6). Spread the washings over the surface of the ten Petri dishes (each dish is incubated from a separate tube) or all the washings over the surface of the Roux flask.

Incubate for 7 d at $30 ^\circ\text{C}$. Wash off the colonies from the Petri dishes with 3,0 ml salt peptone solution (6.6) and the flask with 30,0 ml salt peptone solution (6.6). Bring the suspension over into a sterile flask by using a sterile funnel and close the flask with a sterile stopper.

Heat the solution with occasional shaking, for 30 min in a water bath at approx. $85 ^\circ\text{C}$ in order to kill the vegetative forms. After heating transfer the spore suspension to a sterile centrifuging flask of 40,0 ml and centrifuge for 10 min at 10000 *g*. Eliminate the liquid. Wash the residue with 30,0 ml salt peptone solution (6.6) and centrifuge again. Repeat the washing 3 times. Suspend the spores in 20,0 ml of the salt peptone solution (6.6).

The spore suspension may be stored at $4 ^\circ\text{C}$ to $8 ^\circ\text{C}$ not longer than 4 weeks.

NOTE The spore suspension is also commercially available.