SLOVENSKI STANDARD
oSIST prEN 1104:2017

01-september-2017

Papir, karton in lepenka v neposrednem stiku z živili - Določanje izločanja antimikrobnih snovi

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

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

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

Ta slovenski standard je istoveten z: **prEN 1104**

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

oSIST prEN 1104:2017 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 1104

May 2017

ICS 67.250; 85.060

Will supersede EN 1104:2005

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 les denrées alimentaires - Détermination du transfert des constituants antimicrobiens

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

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 172.

If this draft becomes a European Standard, 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.

This draft European Standard was established by CEN 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 CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. prEN 1104:2017 E

Contents

Page

European foreword

This document (prEN 1104:2017) has been prepared by Technical Committee CEN/TC 172 “Pulp, paper and board”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document will supersede EN 1104:2005.

With regards to EN 1104:2005 the following changes have been made:

a) the definition of the inhibition zone has been clarified;

b) modified Sabouraud nutrient medium for the preparation of *Aspergillus niger* spores has been replaced by 4 % Sabouraud;

c) guidelines for interpreting the results in Annex A have been added, including figures: these guidelines are intended to aid the interpretation of results obtained in the framework of application of EN 1104 standard for the various controls performed and for the samples tested;

d) editorial updating.

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.

NOTE The need of using this Standard can be specified by the legislation regarding paper and board intended to come into contact with foodstuffs.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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)*

EN ISO 7218, *Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations (ISO 7218)*

EN ISO 11133, *Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media (ISO 11133)*

3 Terms and definitions

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

3.1
inhibition zone
obvious area in which growth is absent and which forms around test pieces placed on a nutrient medium inoculated with a preselected test microorganism, due to the release of water-soluble antimicrobial constituents

Note 1 to entry: Proof of the presence of an inhibition zone is provided by the absence of test micro-organism growth (translucent zone) in a minimum of 2 mm width zone at the edges of the test pieces.

4 Principle

A prepared nutrient medium is mixed with an appropriate inoculum and poured into Petri dishes. The test pieces are placed on the nutrient medium before its complete solidification 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 decision.

5 Apparatus

All laboratory equipment and parts of the equipment shall be as described in EN ISO 7218.

5.1 Punch iron:

Diameter = 10 mm to 15 mm, sterilizable.

5.2 Pressing device suitable for pressing the test pieces on the agar plates (e.g. Drigalski spatula).

5.3 Zone reading device (facultative) allowing the measurement of the diameter of the inhibition zone.

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

6 Reagents and media

6.1 General

When water is mentioned in a formula, use distilled water or purified water, as mentioned in EN ISO 11133.

For preparing culture media, general requirements are described in EN ISO 11133. Culture media shall be prepared as follows, or from commercially available dehydrated culture media according to the manufacturer's instructions. Ready-to-use medium may be used when its composition and/or growth performance is comparable to that of formula given hereafter. (according to EN ISO 11133).

6.2 Non ionic wetting agent

For example polyoxyethylenesorbitane monooleate.

6.3 Nutrient medium for the preparation of *Bacillus subtilis* spores

6.3.1 Composition

- Beef extract 3,0 g
- Peptone from casein, tryptic digest 5,0 g
- Sodium chloride, pure 5,0 g
- Agar-agar 12,0 g
- Water 1 000,0 ml

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

6.3.2 Preparation

Dissolve the components, or a dehydrated ready-to-use medium, in the water by mixing while heating.

Separate the nutrient medium into two parts:

- Dispense one part in 300,0 ml portions into 600,0 ml sterile flasks, for example Roux flasks, closed with filter screw 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, for example cellulose stoppers.

Sterilize flasks and test tubes in an autoclave for 15 min at 121 °C. After sterilization, the pH shall be equivalent to 7,2 ± 0,2 when measured at 45 °C. Before solidification, position the test tubes in such a way that the nutrient medium solidifies with a sloping surface.

Medium may be stored according to EN ISO 11133 prescriptions.

6.4 Nutrient medium of Sabouraud for the preparation of *Aspergillus niger* spores

6.4.1 Composition

— Peptone from casein, tryptic digest 5,0 g
— Meat peptone 5,0 g
— D (+) glucose $C_6H_{12}O_6$ H_2O (Dextrose) 40,0 g
— Agar-agar 10,0 g to 15,0 g
— Water 1 000,0 ml

6.4.2 Preparation

Dissolve the components, or a dehydrated ready-to-use medium, in the water by mixing while heating.

Proceed as described in 6.3 by dispensing the medium into sterile flasks or Roux flasks and sterile test tubes.

Sterilize in an autoclave for 15 min at 121 °C. After sterilization, the pH shall be equivalent to 5,6 ± 0,2, when measured at 25 °C.

6.5 Nutrient medium for the inhibition test with *Bacillus subtilis*

6.5.1 Composition

— Peptone from casein, tryptic digest 3,45 g
— Meat peptone 3,45 g
— Sodium chloride, pure 5,1 g
— Agar-agar 13,0 g
— Water 1 000,0 ml

6.5.2 Preparation

Dissolve the components, or a dehydrated ready-to-use medium, in the water by mixing while heating.

Dispense the medium into sterile flasks and close them with filter screw caps.

Sterilize in an autoclave for 15 min at 121 °C. After sterilization, the pH shall be equivalent to 6 ± 0,2, when measured when measured at 45 °C.

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

Medium may be stored according to EN ISO 11133 prescriptions.

6.6 Nutrient medium of modified Sabouraud for the inhibition test with *Aspergillus niger*

6.6.1 Composition

— Peptone from casein, tryptic digest 5,0 g
— Meat peptone 5,0 g
— D (+) glucose $C_6H_{12}O_6$ H_2O (Dextrose) 10,0 g
— Maltose $C_{12}H_{22}O_{11}$ H_2O 10,0 g

— Agar-agar 10,0 g to 15,0 g

— Water 1 000,0 ml

6.6.2 Preparation

Dissolve the components, or a dehydrated ready-to-use medium, in the water by mixing while heating.

Dispense the medium into sterile flasks and close them with filter screw caps.

Sterilize in an autoclave for 15 min at 121 °C. After sterilization, the pH shall be equivalent to 5,4 ± 0,2, when measured at 45 °C.

6.7 Tryptone salt solution

6.7.1 Composition

— Peptone from casein, tryptic digest 1,0 g

— Sodium chloride, pure 8,5 g

— Water 1 000,0 ml

6.7.2 Preparation

Dissolve the components in the water by mixing while heating.

Dispense into flasks or test tubes. Sterilize in an autoclave for 15 min at 121 °C. After sterilization, the pH shall be equivalent to 7,0 ± 0,2, when measured at room temperature.

6.8 Test microorganisms

6.8.1 General

The following strains are used:

Bacillus subtilis DSM 347 (ATCC 6633) and *Aspergillus niger* DSM 1957 (ATCC 6275).

6.8.2 Preparation of working cultures

Working cultures of *Bacillus subtilis* are obtained by inoculating onto the test tubes (6.3.2) and incubating for 7 days at 30 °C ± 2 °C. After incubation the test tubes are stored at 2 °C to 8 °C.

Working cultures of *Aspergillus niger* are obtained by inoculating onto the test tubes (6.4.2) and incubating for 5 days at 25 °C ± 2 °C. After incubation the test tubes are stored at 2 °C to 8 °C.

6.8.3 Preparation of inoculating spore suspension of *Bacillus subtilis*

Transfer aliquots of about 15 ml of the nutrient medium (6.3) cooled to approximately 45 °C to ten sterile Petri dishes (diameter = 90 mm) and allow to solidify.

Wash off the colonies of ten test tubes containing the working solution of *Bacillus subtilis* (6.8.2) with 2,0 ml to 3,0 ml of sterile tryptone salt solution (6.7). Spread the washings over the surface of the ten Petri dishes (each Petri dish is inoculated from a separate tube) or all the washings over the surface of the Roux flask.

Incubate for 7 days at 30 °C. Wash off the colonies from the Petri dishes with 3,0 ml of sterile tryptone salt solution (6.7) or the flask with 30,0 ml of sterile tryptone salt solution (6.7). Bring the suspension into a sterile flask.

Heat the solution with occasional shaking, for 30 min in a water bath at approx. 85 °C in order to kill the vegetative forms. After heating, transfer the spore suspension to a sterile centrifugating flask of 40,0 ml

and centrifuge for 10 min at 10 000 g. Eliminate the liquid. Wash the residue with 30,0 ml of sterile tryptone salt solution (6.7) and centrifuge again.

Repeat the washing 3 times. Suspend the spores in 20,0 ml of the sterile tryptone salt solution (6.7).

The spore suspension may be stored at 2 °C to 8 °C not longer than 4 weeks.

NOTE The spore suspension is also commercially available.

6.8.4 Preparation of inoculating spore suspension of *Aspergillus niger*

Transfer aliquots of about 15 ml of the liquefied Sabouraud medium (6.4), cooled to approx. 45 °C to at least 5 sterile Petri dishes (d = 90 mm) and allow to solidify.

With an inoculation loop, inoculate the *Aspergillus niger* strain from the working cultures (6.8.2) onto the Petri dishes. Each Petri dish is inoculated from a separate tube. The flask is inoculated from at least five test tubes.

Incubate for 8 to 10 days at 25 °C ± 2 °C. Transfer the conidia with an inoculating ring moistened with the tryptone salt solution (6.7) to a sterile test tube containing 10,0 ml of tryptone salt (6.7) mix with 0,01 ml of a non ionic wetting agent (6.2) and seal with a sterile stopper.

Shake the suspension well before using. The inoculation suspension may be stored at 2 °C to 8 °C not longer than 4 weeks.

NOTE The spore suspension is also commercially available.

6.8.5 Concentrations of spores for inhibition test

Dilute the spore suspension such that the concentration of spores in the test agar is:

— *Bacillus subtilis*: 10^4 spores per ml test agar;

— *Aspergillus niger*: 10^5 conidia per ml test agar.

Measure the spore density of the inoculation suspensions of *Bacillus subtilis* (6.8.3) and *Aspergillus niger* (6.8.4) by the method of enumeration on solid medium (EN ISO 7218) by using nutrient media described in 6.5 and 6.6.

6.9 Positive controls

6.9.1 Antibiotic

Penicillin G 0,03 units (commercially available).

6.9.2 Antifungal agent

Use:

— An isothiazolinon solution: mix of 5-chloro-2-methyl-4-isothiazolin-3-on and 2-methyl-4-isothiazolin-3-on with a concentration of active substance of 2,5 % (diluted 1: 100).

Or

— An amphotericin B 20 µg solution.

NOTE These solutions are commercially available.