SLOVENSKI STANDARD
SIST EN 1104:1997

01-september-1997

Papir, karton in lepenka v neposrednem stiku z ģivili - Doloļ anje izloļ anja antimikrobnih snovi

Paper and board intended to come into contact with foodstuff - Determination of transfer of antimicrobic constituents

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

Papier et carton destinés a entrer en contact avec des denrées alimentaires - Détermination du transfert des consituants anti-microbiens

Ta slovenski standard je istoveten z: **EN 1104:1995**

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

SIST EN 1104:1997 **en**

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English version

Paper and board intended to come into contact with foodstuff - Determination of transfer of antimicrobic constituents

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart,36 B-1050 Brussels

Foreword

This European Standard has been prepared by the 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of an identical text or by endorsement, at the latest by April 1996, and conflicting national standards shall be withdrawn at the latest by April 1996.

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

1 Scope

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

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.

prEN ISO 186 Paper and board - Sampling to determine average quality (ISO 186:1994)

3 Definition

For the purposes of this European Standard the following definition applies:

Inhibition zone: A zone formed when paper or board placed on nutrient medium which has been seeded with a preselected test organism releases water soluble antimicrobic constituents.

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 antimicrobic constituents.

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

5 Apparatus

5.1 Punch iron, d = 10 mm to 15 mm, sterilizable.

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

5.3 Zone reading device, to measure diameter of inhibition.

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

5.4 Spectrometer, for use at the wavelength of 546 nm, with cells of an optical path length of 10 mm.

NOTE: The spectrometer is not compulsory.

5.5 Ordinary microbiological laboratory apparatus

6 Reagents

6.1 Water, freshly destilled 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

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

Prepare the nutrient medium as follows:

Dissolve the components, or a ready made medium of a comparable composition, in water by boiling. Adjust the pH to (7,2 ± 0,2) as required. Separate the nutrient medium into two parts. Dispense one part in 300,0 ml portions into nutrient medium flasks or 600,0 ml Roux flasks and stopper them with 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 cellulose stoppers.

Sterilize flasks and test tubes for 15 min at (121 ± 1)° C. After sterilization 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 for not longer than 14 days.

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

Cool the Roux flasks to solid.

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) 3,0 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

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

Prepare the Sabouraud modified mould nutrient medium as follows:

Dissolve the components, or a ready made medium of a comparable composition, by boiling. Adjust the pH to (5,4 ± 0,1) as required. Proceed as described in 6.3 dispensing the medium into nutrient medium flasks or Roux flasks and test tubes and the sterilizing and cooling procedures.

6.5 Nutrient medium for inhibition test with Bacillus subtilis

A typical formulation of the nutrient medium for inhibition test with Bacillus subtilis is:

- 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

The pH of the ready made nutrient medium shall be (6,0 ± 0,1) referred at a temperature of 45° C.

Prepare the nutrient medium as follows:

Dissolve the components, or a ready made medium of a comparable composition, in water by boiling. Adjust the pH to (6,0 ± 0,1) as required.

Dispense 300,0 ml portions into nutrient medium flasks and stopper them with Kaspenberg caps and sterilize for 15 min at (121 ± 1)° C.

Cool the flasks to below 60° C for the preparation of the inoculation medium (8.2.1) 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 Kaspenberg caps and sterilize for 15 min at (121 ± 1)° C.

The solution shall be used within 8 days if stored at room temperature and within 14 days when stored at 4° C to 8° C.

6.7 Test micro-organisms

The following are used:

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

Working cultures of B. subtilis are obtained by inoculating onto the test tubes (6.3) and incubating for 7 days at 30° C. After incubation the test tubes are stored at 4° C to 8° C.

Working cultures of A. niger are obtained by inoculating onto the test tubes (6.4) and incubating for 5 days at 25° C. After incubating the test tubes are stored at 4° C to 8° C.

6.7.1 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° C, to 10 sterile Petri dishes (d = 90 mm) and allow to solidify.

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

Wash off the colonies of 10 test tubes with the working culture of B. subtilis (6.7) with 2,0 ml to 3,0 ml sterile salt peptone solution (6.6). Spread the washings over the surface of the 10 Petri dishes (each dish is incubated 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 slt peptone solution (6.6) and the Roux 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 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° C to 8° C for not longer than 4 weeks.

NOTE : The spore suspension is also commercially available.

6.7.2 Preparation of inoculating spore suspension of Aspergillus niger

Transfer aliquots of about 15,0 ml of the liquefied modified Sabouraud medium (6.4), cooled to approximately 45° C to at least 5 sterile Petri dishes (d = 90 mm) and allow to solidify.

The nutrient medium in Roux flasks (6.4) is ready for inoculation.

Inoculate the A.niger strain from the working cultures (6.7) onto the Petri dish. Each Petri dish is inoculated from a separate tube. The Roux flask is incubated from at least 5 test tubes.

Incubate for 8 to 10 days a 25° C. Transfer the conidia with an inoculating ring moistened with salt peptone solution (6.6) to a sterile test tube containing 10,0 ml of salt peptone solution (6.6) mixed with 0,01 ml of a non ionic wetting agent (6.2) and seal with a sterile stopper.

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

6.7.3 Concentrations of spores for inhibition test

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

B.subtilis: 10^4 spores per ml test agar
A.niger: 10^5 conidia per ml test agar

Measure the spore density of the inoculation suspension of B.subtilis (6.7.1) by the conventional plate counting method on nutrient medium (6.3) or spectrometrically. The transmission of the spore suspension is measured at 546 nm using salt peptone solution (6.6) as a reference. Adjust the transmission with the salt peptone solution to 80 %.

Determine the spore density of the inoculation suspension of A.niger (6.7.2) using the conventional plate counting method on nutrient medium (6.4).

7 Sampling and preparation of test pieces

Sample in accordance with prEN ISO 186. Touch only the edges of the samples. Lay the samples immediately one above the other in a sterile sample vessel or wrap the samples in aluminium foil.

Take at least 10 samples per unit. Punch at least 20 circular test pieces for each microorganism out of the samples with a sterile punch iron (5.1). Transfer the test pieces immediately into a sterile vessel. Touch the test pieces only with sterile forceps or tweezers.

8 Procedure

8.1 Sterilization

Sterilize the forceps or tweezers, punch iron, centrifuging flasks (packed in aluminium foil), conical flasks, test tubes with cellulose stoppers and vessels in the autoclave for 15 min at (121 ± 1)° C. Sterilize volumetric pipettes made of glass in pipette boxes in the hot air sterilizer for 2 h at (180 ± 1)° C.

8.2 Preparation of plates

Prepare at least 3 Petri dishes for each test.

8.2.1 Bacillus subtilis

Liquefy sterile test agar (6.5). Cool to below 60° C. Add an amount of the inoculating suspension (6.7.1) resulting in a density of 10^4 spores per ml test agar. Distribute the suspension evenly by careful shaking. Dispense aliquots of about 15,0 ml into each sterile Petri dish (d = 90 mm). Lay three test pieces on the still semi solid nutrient medium using sterile forceps or tweezers. Press the test pieces down slightly with a suitable sterile device (5.2), ensuring that no air cushions are formed.

The side intended to come into contact with foodstuffs should be placed face-downwards. Otherwise both sides shall be tested.

Prepare for each analysis a control nutrient agar plate without test pieces.

8.2.2 Aspergillus niger

Prepare plates as described in 8.2.1, but in this case with Sabouraud modified nutrient medium (6.4). This suspension should be used as the test agar and as inoculating suspension of A.niger (6.7.2). A density of 10^5 conidia per ml test agar should be reached.

8.3 Incubation

The Petri dishes prepared as in 8.2.1 and 8.2.2 are stored for 2 h in a refrigerator at 4° C to 8° C to facilitate prediffusion.

Incubate Petri dishes prepared as in 8.2.1 and 8.2.2 at 30° C and 25° C respectively.

Arrange the plates in the refrigerator and in the incubator in such a way that the cover of the Petri dish is underneath preventing condensation water dripping onto the sample.

9 Evaluation

Evaluate the tests with the bacterium or the fungus after 3 and 5 days respectively.

NOTE 1: A preliminary inspection of the Petri dishes after 1 and 2 days is useful.

Samples without evidence of an inhibition zone area are considered to contain no water soluble antimicrobic substances.

NOTE 2: Samples overgrown are evaluated as free of inhibition zone.

NOTE 3: The diameter of the inhibition zone may be measured with a magnifying glass and a measuring scale.

NOTE 4: If the control nutrient agar plates exhibit no growth the test is repeated with new inoculating suspension.

10 Test report

The following shall be stated in the test report, referring to this European Standard and any relevant specifications used:

a) Date and place of testing;

b) Identification of material tested;

c) The results;

d) Any deviations from this standard;

e) Any other circumstances that may have affected the result.