SLOVENSKI STANDARD
SIST EN 1650:2008/kFprA1:2012

01-november-2012

Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje fungicidnega delovanja ali delovanja kemičnih razkužil in antiseptikov na kvasovke v živilski in drugih industrijah, gospodinjstvu in javnih ustanovah - Preskusna metoda in zahteve (faza 2, stopnja 1) - Dopolnilo A1

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der fungiziden oder levuroziden Wirkung chemischer Desinfektionsmittel und Antiseptika in den Bereichen Lebensmittel, Industrie, Haushalt und öffentliche Einrichtungen - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité fongicide ou levuricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine de l'agro-alimentaire, dans l'industrie, dans les domaines domestiques et en collectivité - Méthode d'essai et prescriptions (phase 2, étape 1)

Ta slovenski standard je istoveten z: **EN 1650:2008/FprA1**

ICS:

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

SIST EN 1650:2008/kFprA1:2012 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

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FINAL DRAFT
EN 1650:2008

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English Version

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

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This draft amendment is submitted to CEN members for unique acceptance procedure. It has been drawn up by the Technical Committee CEN/TC 216.

This draft amendment A1, if approved, will modify the European Standard EN 1650:2008. If this draft becomes an amendment, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for inclusion of this amendment into the relevant national standard without any alteration.

This draft amendment 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.

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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.

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COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 1650:2008/FprA1:2012) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This document is currently submitted to the Unique Acceptance Procedure.

1 Modification to 5.2.2.3, Malt extract agar (MEA)

Replace the whole sub-clause 5.2.2.3 with the following:

"Malt extract agar, consisting of:

Malt extract	30,0 g
Agar	15,0 g
Water (5.2.2.2)	to 1 000,0 ml

The malt extract should be food grade (e.g. Cristomalt powder from Difal) or equivalent that is not highly purified and not only based on maltose (e.g. Malt extract from OXOID) [1)]. However if there are problems producing at least 75 % spiny spores see 5.4.1.4.2.

Sterilize in the autoclave [5.3.2.1a)]. After sterilization, the pH of the medium shall be equivalent to 5,6 ± 0,2 when measured at (20 ± 1) °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 MEA. Annex B gives guidance on the neutralizers that may be used."

2 Modification to 5.3.2, Usual microbiological laboratory equipment[2)] and in particular, the following:

Replace the whole sub-clause 5.3.2 with 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 $(180\ ^{+5}_{0})$°C for a minimum holding time of 30 min, at $(170\ ^{+5}_{0})$ °C for a minimum holding time of 1 h or at (160^{+5}_{0})°C for a minimum holding time of 2 h.

5.3.2.2 Water baths, capable of being controlled at (20 ± 1) °C, at (45 ± 1) °C (to maintain melted MEA in case of pour plate technique) and at additional test temperatures ± 1 °C (5.5.1).

5.3.2.3 Incubator, capable of being controlled at (30 ± 1) °C.

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0,1 pH units at (20 ± 1) °C.

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1) 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. Equivalent products may be used if they can be shown to lead to the same results.

2) Disposable sterile equipment is an acceptable alternative to reusable glassware.

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 Shaker

a) Electromechanical agitator, e.g. Vortex® mixer [3].

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 µm pore size for sterilization of hard water (5.2.2.7), bovine albumin (5.2.2.8.2 and 5.2.2.8.3) and sucrose (5.2.2.8.6), and if the membrane filtration method is used (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 °C to 8 °C.

5.3.2.9 Graduated pipettes, of nominal capacities 10 ml and 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

5.3.2.13 Fritted filter, with porosity of 40 µm to 100 µm according to ISO 4793.

5.3.2.14 Flasks with ventilated caps.”

3 Modification to 5.4.1.3.2, *Aspergillus niger* (mould)

Replace the whole sub-clause 5.4.1.3.2 with the following:

“

5.4.1.3.2 *Aspergillus* brasiliensis (previously *A. Niger*) **(mould)**

For *Aspergillus brasiliensis* (previously *A. Niger*) (5.2.1), use only the first subculture grown on MEA (5.2.2.3) in Petri dishes or flasks with ventilated caps (5.3.2.14) and incubate for 7 d to 9 d. No further subculturing is needed. Do not stack the Petri dishes during the incubation to improve the temperature homogenization.

At the end of incubation, all the cultures have to show a dark brown or black surface. Cultures with appearance of rare and small white or grey areas might be kept (see Figure 1).

[3] Vortex® is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.

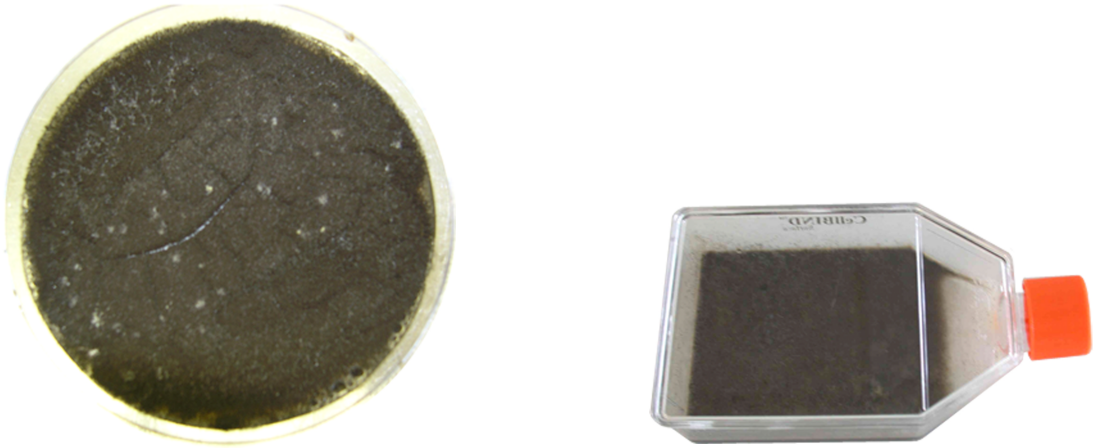

Figure 1 — Photos N°1: *A. brasiliensis* ATCC 16404 after 7 d of incubation at 30 °C (right cultures)

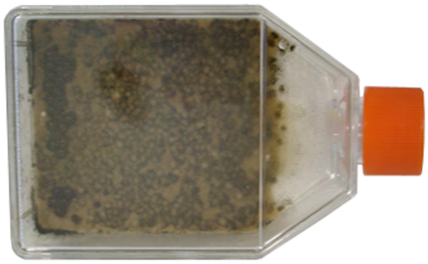

Figure 2 — Photo N°2: *A. brasiliensis* ATCC 16404 after 7 d of incubation at 30 °C (wrong culture)”

4 Modification to 5.4.1.4.2, *Aspergillus niger*

Replace the whole sub-clause 5.4.1.4.2 with the following:

“

5.4.1.4.2 *Aspergillus brasiliensis* (previously *A. niger*) **(mould)**

The procedure for preparing the *Aspergillus brasiliensis* test suspension is as follows.

a) Take the working culture (5.4.1.3.2) and suspend the spores in 10 ml of sterile 0,05 % (w/v) polysorbate 80 solution in water (5.2.2.2). Using a glass rod or spatula, detach the conidiospores from the culture surface. Transfer the suspension into a flask and gently shake by hand for one minute together with 5 g of glass beads (5.3.2.11). Filter the suspension through a fritted filter (5.3.2.13).

b) Carry out a microscopic examination under x 400 magnification immediately after the preparation to show:

   1) the presence of a high concentration (at least 75 % of spiny spores) of characteristic mature spores i.e. spiny spores (versus smooth spores) [see Figure 3 and Figure 4];

2) the absence of spore germination (check at least ten fields of view);

3) if germinated spores are present, discard the suspension;

4) the absence of mycelia fragments (check at least ten fields of view).

If mycelia are present, proceed to a 2nd fritted filtration.

If mycelia are still present, discard the suspension.

NOTE 1 If 75 % spiny spores are not achieved it may be due to the *Aspergillus brasiliensis* culture or the media used to produce these spores. In this situation it will be necessary to obtain the culture from another culture collection and/or use a MEA from a different supplier.

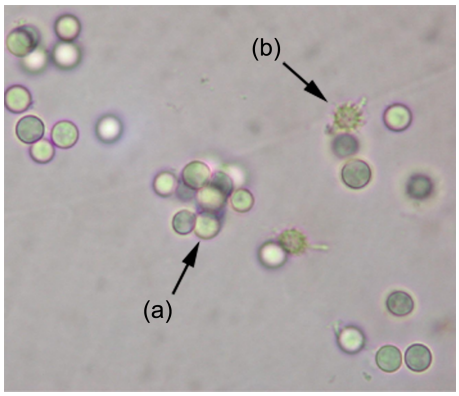

Figure 3 — Photo N°3 Observation of conidiospores under light microscope: presence of smooth (a) and spiny (b) spores (wrong suspension)

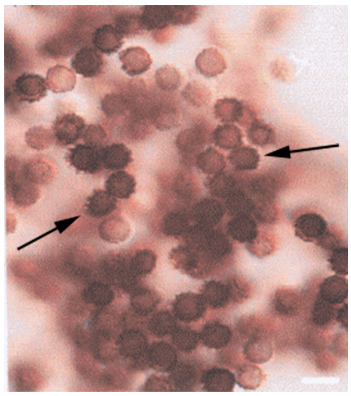

Figure 4 — Photo N°4 Observation of conidiospores under light microscope: High concentration of characteristic mature spores with spiny aspect (right suspension)

c) Adjust the number of spores in the suspension to 1,5 x 10^7 cfu/ml to 5,0 x 10^7 cfu/ml using the diluent (5.2.2.4), estimating the number of cfu by any suitable means. Use the suspension within 4 h in a water bath controlled at 20°C ± 1°C (5.3.2.2). In any case, adjust the temperature according to 5.5.1.4 only immediately before the start of the test (5.5.2 or 5.5.3).

NOTE 2 The use of a cell counting device for adjusting the number of cells is highly recommended. When using a suitable counting chamber, it is essential to follow the instructions explicitly.

Each laboratory should therefore produce calibration data to establish the relationship between the counts obtained using the counting device and the counts (5.4.1.6) obtained by the pour plate or the spread plate technique. Experienced laboratories found a better fit to the required number of spores when the spore suspension count in the device was 10 % to 50 % higher than the number aimed at.

d) For counting, prepare 10^{-5} and 10^{-6} 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 thespread plate technique.