BS EN 15457:2014

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Paints and varnishes — Laboratory method for testing the efficacy of film preservatives in a coating against fungi

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee STI/28, Paint systems for non-metallic substrates.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Paints and varnishes - Laboratory method for testing the efficacy of film preservatives in a coating against fungi

Peintures et vernis - Méthode d'essai en laboratoire permettant de déterminer l'efficacité des préservateurs du feuil d'un revêtement contre les champignons

Beschichtungsstoffe - Laborverfahren für die Prüfung der Wirksamkeit von Filmkonservierungsmitteln in einer Beschichtung gegen Pilze

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Foreword

This document (EN 15457:2014) has been prepared by Technical Committee CEN/TC 139 “Paints and varnishes”, 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of an identical text or by endorsement, at the latest by February 2015 and conflicting national standards shall be withdrawn at the latest by February 2015.

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Introduction

This document identifies criteria to assess efficacy of film preservatives in a coating against fungi. The results of the method allow evaluation of an active substance with regard to its inclusion in Annex I of the Biocidal Products Directive 98/8/EC (Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market – BPD) or in the list of the Biocidal Product Regulation (BPR, Regulation (EU) 528/2012).

The characteristics of the biocide treated coating material should conform to national regulations with regard to health, safety and the environment.

1 Scope

This European Standard specifies a laboratory test method for determining the biocidal/biostatic efficacy of single active substances or combinations thereof used in film preservatives in a coating against fungal growth. This standard does not apply to coatings not susceptible to fungal growth. The test method comprises only active substances for film preservation, not the protection of the substrate itself, e.g. wood, which is dealt with in another standard. The test method is applicable for active substances used for wood and masonry coatings. It is not applicable to marine coatings.

Safety, health and environmental aspects are not in the scope of this standard.

Determination of the performance of film preservatives in coatings by applying ageing procedures is not within the scope of this standard.

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 12469, *Biotechnology - Performance criteria for microbiological safety cabinets*

EN 16492:2014, *Paints and varnishes - Evaluation of the surface disfigurement caused by fungi and algae on coatings*

EN 23270, *Paints and varnishes and their raw materials - Temperatures and humidities for conditioning and testing (ISO 3270)*

EN ISO 1513, *Paints and varnishes - Examination and preparation of test samples (ISO 1513)*

3 Terms and definitions

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

3.1
active substance
substance or micro-organism that has an action on or against harmful organisms

[SOURCE: Biocidal Product Regulation (BPR, Regulation (EU) 528/2012), Article 3.1 (c), modified – the article "a" between "or" and "micro-organism" was deleted]

4 Principle

To determine the fungicidal efficacy of film preservatives in a coating, the coating material is applied to a substrate conditioned according to EN 23270, placed onto an agar surface, inoculated with a standard fungal spore suspension and incubated. Conclusions can be drawn to the fungicidal efficacy of the film preservatives in a coating from the intensity of the fungal growth on the surface of the specimen after incubation. The method described here is a semiquantitative, comparative method between coatings, with and without film preservatives.

5 Apparatus and materials

5.1 Cutting device for preparing the specimens (coated filter paper with a diameter of 55 mm).

5.2 Autoclave

5.3 Incubator capable of maintaining (24 ± 2) °C.

5.4 Pipette, in the range between 100 µl to 1 000 µl, with sterile tips or combi-tips of 12,5 ml.

5.5 Filter paper without fungicidal effect (e.g. cellulose with a pore size of 0,45 µm and a thickness of 650 µm).

5.6 Automatic welding apparatus to seal the bags.

5.7 Sterilized glass bottles (100 ml).

5.8 Laboratory balance, capable of weighing to an accuracy of 0,1 g.

5.9 Microscope

5.10 Device to determine cell count (commercially available counting chamber, e.g. Thoma chamber).

5.11 Wetting agent (e.g. N-Methyltaurine).

5.12 Device for applying the coating

5.13 Sterilized test tubes or **other sterilized glassware** for preparing slant agar cultures.

5.14 Sterile Drigalski spatula

5.15 Sterile platinum loop

5.16 Sterile glass funnel with cotton wool

5.17 Sterile Petri dishes (with a diameter of 94 mm, and a height of 16 mm).

5.18 Sterile tweezers

5.19 Sterile water

5.20 Class 2 microbiological safety cabinet according to EN 12469.

6 Fungi

6.1 Fungi more likely to grow in an exterior environment

a) *Aureobasidium pullulans* (DSM[1)] 2404)

b) *Alternaria alternata* (DSM 62010)

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1) DSM = DSMZ = Deutsche Sammlung für Mikroorganismen und Zellkulturen (German collection of micro organisms and cell cultures), Braunschweig, Germany.

c) *Cladosporium cladosporioides* (DSM 62121)

d) *Phoma violaceae* (IMI[2)] 49948ii)

e) *Ulocladium atrum* (IMI 79906 or DSMZ 63068)

6.2 Fungi more likely to grow in an interior environment

a) *Aspergillus versicolor* (DSM 1943)

b) *Aspergillus niger* (DSM 12634)

c) *Stachybotrys chartarum* (DSMZ 2144)

d) *Penicillium purpurogenum* (DSM 62866)

e) *Rhodotorula mucilaginosa* (DSM 70825)

The spore suspension used for the test shall be a mixture containing two fungi selected from the first group (6.1) and two fungi selected from the second group (6.2).

7 Sampling and preparation of test samples and of specimens

7.1 Sampling

Take a representative sample of the coating material or of the coating system for testing in accordance with EN ISO 1513.

7.2 Preparation of test samples (see Annex A)

Coat a strip of filter paper without biocidal effect with the coating material to be tested. The application rate shall be that recommended by the coating manufacturer for normal use.

7.3 Conditioning of the test samples

Condition the test sample in a horizontal position for at least 5 days at (23 ± 2) °C and (50 ± 5) % relative humidity, in accordance with EN 23270.

NOTE The conditioning time might vary according to the coating material and end use corresponding to information given by the manufacturer.

7.4 Preparation and number of specimens

After conditioning, three specimens each with a diameter of 55 mm shall be prepared from the test samples. The specimens shall be sealed in a plastics bag and sterilized using gamma radiation of ≥ 10 kGy. Other methods of sterilization may be agreed between the parties.

For each test series three specimens coated with coating material containing the film preservative, three specimens coated with the same coating material without film preservative and three specimens of the uncoated substrate shall be tested.

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2) IMI = CABI = Bioscience Genetic Resource Collection, Egham, UK.

8 Procedure

8.1 Preparation of the Petri dishes with the culture medium

A malt (3 %)-agar(1,5 %)-medium shall be sterilized in the autoclave. After cooling the medium to 55 °C to 60 °C, 20 ml shall be poured into each sterile Petri dish under aseptic conditions.

8.2 Preparation of stock cultures and sub-cultures

Sub-cultures shall be obtained by inoculating spore material from a stock culture to freshly prepared agar slope culture media and shall be used for preparing the spore suspension. From these sub-cultures further sub-cultures can be derived in sufficient number. After the inoculation the sub-cultures shall be incubated at (24 ± 2) °C until good sporulation has been achieved. This might require 3 days to 7 days, depending on the fungal species used for testing. The sub-cultures can be stored satisfactorily at 3 °C to 7 °C for a period of 3 months.

8.3 Preparation of the spore suspension

For preparing the spore suspension a well-sporulating sub-culture is used. To this sub-culture 5 ml of sterile deionized water should be added (if required also add a surfactant – e.g. 0,1 % N-Methyltaurine). The spores shall be carefully washed down from the agar slope, using a platinum loop as an aid, filtered through a sterile glass funnel with cotton wool and collected in a sterile glass bottle.

After counting the spore concentration by using a commercially available counting chamber and diluting each with sterile water to 10^6 to 10^7 spores/ml all spore suspensions intended for testing shall be mixed in equal parts.

8.4 Inoculation and incubation (see Annex A)

In addition to the coated and uncoated specimens (see 7.4) a further three Petri dishes containing the nutritive agar medium only shall be inoculated. The sterilized specimens shall be placed centrally onto the surface of the culture media using sterile tweezers. The coated surface of the specimen shall be face up and there shall be full contact without air bubbles between the specimens and the surface of the culture medium.

Should the coating lead to an undulation of the filter paper the paper should be kept even and in close contact with the agar by appropriate means. Otherwise a different substrate may be used. Check that the substrate does not inhibit growth of each selected test organism under the test conditions. If the substrate does inhibit growth it cannot be used.

Under aseptic conditions in a safety cabinet the specimens shall be uniformly inoculated with 0,2 ml each of the mixed spore suspension using a suitable pipette. It is permissible to use up to an additional 0,8 ml of diluent to ensure an even distribution over the surface. Afterwards the suspension is spread out onto the agar plate with Drigalski spatula. The surface of the specimen shall remain free of agar medium during this operation.

Following inoculation incubate the agar plates at (24 ± 2) °C.

8.5 Assessment

Assess the fungal growth 7 days, 14 days and 21 days after the inoculation, using the assessment scale EN 16492:2014, Table A.3 (see also Annex B).The assessment is carried out visually macroscopically.

NOTE 1 If required a microscope can be used, in order to exclude contamination by foreign materials.

The maximum duration of the test shall be 21 days. However, testing may be considered complete at an earlier stage provided that the unpreserved specimens have a fungal growth rating of “4”. At the same time preserved specimens shall be rated. The duration of test selected by the test laboratory for the assessment shall be recorded.

The test is considered to be valid if the samples without film preservatives are rated “4”.

The test shall be rejected and repeated, if:

— contamination by other microorganisms occurs to such an extent that they interfere with the assessment;

— specimens without biocide show no fungal growth;

— uncoated and sterilized substrates show no fungal growth.

NOTE 2 It is considered that for the purpose of this test the efficacy of film preservatives in the coating is demonstrated if the specimens containing film preservatives are rated less than “4”.

9 Test report

The test report shall include at least the following information:

a) microorganisms used and cell count applied in the test;

b) active substance(s) and concentration;

c) details necessary to identify the product tested;

d) reference to this European Standard (EN 15457:2014);

e) nature and the dimensions of the substrate (see Clause 4, 7.2 and 7.4);

f) number of coats and the method of application of the coating or coating system including waiting times and spreading rates;

g) method and extent of conditioning before testing;

h) test temperature;

i) validity of the test;

j) result and rating of each specimen;

k) any deviation from the test method specified;

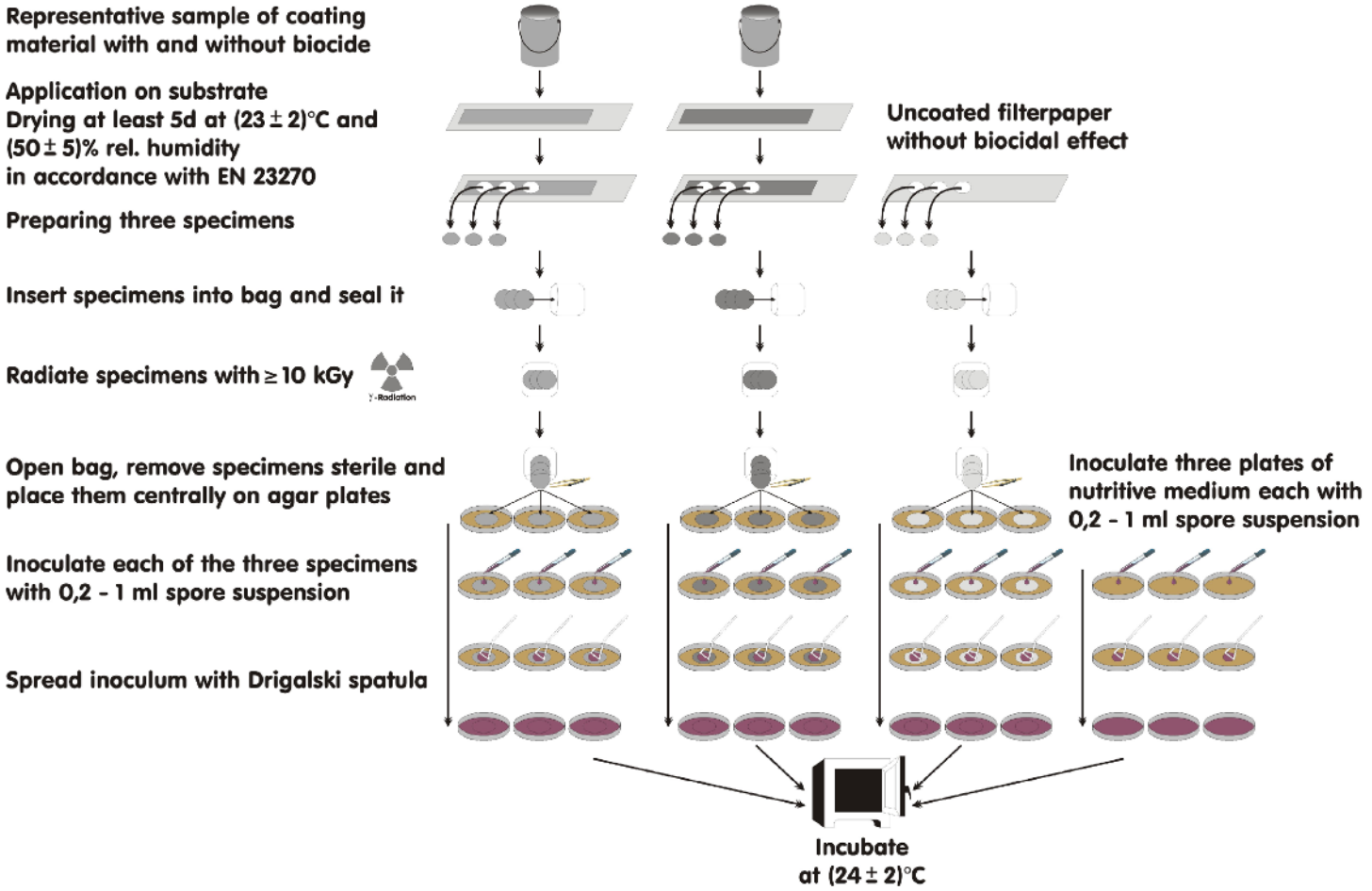
l) any unusual features (anomalies) observed during the test;

m) date of the test.

NOTE The interpretation and practical conclusions that can be drawn from a test report demand a specialized knowledge of the subject of film preservatives and, for this reason, this test report cannot of itself constitute an approval certificate.

Annex A
(informative)

Laboratory method for testing the efficacy of film preservatives in a coating against fungi



Annex B
(informative)

Designation of the percentage area of disfigurements according to EN 16492:2014, Table A.3

Table B.1 — Rating scheme for designating the percentage area of disfigurements

Rating	Percentage area of disfigurements
0	no growth on the surface of the specimen
1	up to 10 % growth on the surface of the specimen
2	more than 10 % up to 30 % growth on the surface of the specimen
3	more than 30 % up to 50 % growth on the surface of the specimen
4	more than 50 % up to 100 % growth on the surface of the specimen

Bibliography

[1] Biocidal Product Regulation (BPR, Regulation (EU) 528/2012)

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