BS EN 15458:2014

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

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

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ISBN 978 0 580 84803 2

ICS 87.040

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2014.

Amendments issued since publication

Date	Text affected

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15458

August 2014

ICS 87.040

Supersedes EN 15458:2007

English Version

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

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

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

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Foreword

This document (EN 15458:2014) 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of an identical text or by endorsement, at the latest by February 2015 and conflicting national standards shall be withdrawn at the latest by February 2015.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 the efficacy of film preservatives in a coating against algae. 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 algal growth. The standard does not apply to coatings not susceptible to algal 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 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

For the determination of the algicidal 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 algal suspension and incubated over a certain period of time under conditions appropriate for algal growth. Conclusions can be drawn with regard to the algicidal efficacy of the film preservatives in a coating from the intensity of the algal growth on the coated surface of the specimen after incubation. The method described in this standard 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** for sterilization.

5.3 **Incubator,** capable of maintaining (23 ± 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 biocidal 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, 0,5 l, 1 l).

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

5.9 Bold modified Basal medium as specified in the method (see 8.1).

5.10 Stock solution (see 8.2).

5.11 Culture flask with cap (0,5 l or 1 l).

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

5.13 Microscope

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

5.15 Device for applying the coating

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

5.17 Sterile tweezers

5.18 Sterile water

5.19 Class 1 microbiological safety cabinet according to EN 12469.

5.20 Luxmeter

5.21 Cold white or **daylight lamp**

6 Microorganisms

— Blue-green algae *Nostoc commune* SAG[1)] 1453-3;

— Blue-green algae *Gloeocapsa atrata* Kützing (syn. *Anacystis montana*) CCAP[2)] 1430/1;

— Green algae *Klebsormidium flaccidum* SAG 335-5*;*

— Green algae *Stichococcus bacillaris* SAG 379-1a = CCAP 379/1A.

From these four microorganisms one blue-green and one green algae are selected.

7 Sampling and preparation of test samples and of specimens

7.1 Sampling

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

7.2 Preparation of test samples (see Annex A)

1) SAG = (Sammlung von Algenkulturen (Culture Collection of Algae), Göttingen; available at: Georg-August Universität Göttingen, Germany.

2) CCAP = Culture Collection of Algae and Protozoa; SAMS Research Services Ltd, Scottish Marine institute Oban, Scotland, UK.

Coat a strip of filter paper without biocidal effect with the coating material/system 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 samples 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 of 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 upon 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.

8 Procedure

8.1 Preparation of Bold's Basal Medium [3)]

For the algal nutritive solution the following substances are required:

a) 10 ml each of stock solutions a) to f) in 8.2;

b) 1 ml each of trace element stock solutions g) to j) in 8.2;

c) 940 ml demineralized or distilled water;

d) 15 g agar (only for the solid nutritive medium).

The solution shall be sterilized in the autoclave. For the test both solid (with 1,5 % agar) and also liquid nutritive medium are required.

8.2 Preparation of the stock solutions

Stock solutions:

a)	$NaNO_3$	10,0 g	Distilled water	400 ml
b)	$CaCl_2 \cdot 2H_2O$	1,0 g	Distilled water	400 ml
c)	$MgSO_4 \cdot 7H_2O$	3,0 g	Distilled water	400 ml
d)	K_2HPO_4	3,0 g	Distilled water	400 ml
e)	KH_2PO_4	7,0 g	Distilled water	400 ml
f)	NaCl	1,0 g	Distilled water	400 ml

Trace element stock solutions:

g)	Ethylenediaminetetraacetic acid	50 g		
	KOH	31 g	Distilled water	1 000 ml
h)	$FeSO_4 \cdot 7H_2O$	4,98 g	Distilled water (acidified)	1 000 ml

3) Bischoff, H. W. & Bold, H. C. (1963): Phycological Studies. IV. Some soil algae from Enchanted Rock and related algal species. – Univ. Texas Publ. 6318: p. 1-95

	(acidified distilled water = 1 ml concentrated H_2SO_4 in 999 ml distilled water)			
i)	H_3BO_3	11,42 g	Distilled water	1 000 ml
j)	$ZnSO_4 \cdot 7H_2O$	8,82 g	Distilled water	1 000 ml
	MoO_3	0,71 g		
	$Co(NO_3)_2 \cdot 6H_2O$	0,49 g		
	$MnCl_2 \cdot 4H_2O$	1,44 g		
	$CuSO_4 \cdot 5H_2O$	1,57 g		

8.3 Preparation of the Petri dishes with culture medium

After cooling to about 55 °C to 60 °C, pour the algal nutritive agar into sterile Petri dishes. To examine the films, the Petri dishes shall be filled with the usual quantity (about 20 ml) of the medium. For thick coatings (e.g. renders) the Petri dishes should be filled with a thin layer (about 2 mm) of nutritive agar only.

8.4 Preparation of stock cultures and sub-cultures

Stock cultures:

Sterile test tubes with agar slant shall be prepared. Depending on the size of the test tube 10 ml to 20 ml of Bold's Basal algal nutritive agar shall be poured into test tubes, closed, sterilized, stored in an inclined position under sterile conditions and allowed to become solid. From the original cultures (*Nostoc commune* SAG 1453-3; *Gloeocapsa atrata* Kützing (syn. *Anacystis montana*) CCAP 1430/1, *Klebsormidium flaccidum* SAG 335-5, *Stichococcus bacillaris* SAG 379-1a = CCAP 379/1A) sub-cultures shall be prepared on agar slants for use as stock cultures. The incubation takes place at room temperature and under illumination, using a cycle of 16 h illumination and 8 h darkness. Fluorescent tubes of the type daylight or white light shall be used, at a distance of about 50 cm at about (1 000 ± 200) lx.

Alternatively, Bold's Basal Medium (according to 8.1) without agar can be used to prepare liquid cultures.

NOTE 1 From experience it is known that the stock cultures will have grown in about 2 weeks to such an extent that sub-cultures can be prepared from them.

Sufficient stock cultures should always be kept in reserve.

Sub-cultures:

From the stock cultures sub-cultures shall be prepared in conical flasks containing liquid nutritive medium, followed by incubation as described above.

NOTE 2 Experience shows that these sub-cultures will have grown after 7 days to 14 days, to such an extent that they can be used further. Cultures with filamentous algae can be shaken up somewhat more frequently, to loosen and disintegrate the filaments.

8.5 Preparation of the algal suspension

Before starting the tests, 200 ml of an actively-growing, ready-to-use individual algal culture shall be mixed with 200 ml of sterile algal nutrient solution. These two components shall be mixed thoroughly, but without introducing contamination, so as to disperse cells and break-up filaments. The resulting suspension should be slightly coloured and contain approximately 10^6 cfu/ml[4)]. To obtain a mixture of different algal species for test use, combine the required individual algal cultures with sterile algal nutrient solution in the above proportions (e.g. two different test species will require 2 × 200 ml algal suspension plus 2 × 200 ml sterile algal nutrient solution = 800 ml total).

4) cfu = colony forming units.

8.6 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 medium only shall be inoculated.

In a safety cabinet the sterilized specimens shall be placed centrally onto the solid algal nutritive medium in the Petri dishes. The coated surface of the specimen shall be face up and there shall be full contact without air bubbles between the specimen and the surface of the culture medium. Coat the specimens with a layer (1 mm thick) of the algal suspension (using a sterile pipette). The distribution of the algae in the suspension should be as uniform as possible (shaking up the suspension before application). Ensure that the specimen is completely covered with the nutritive solution and that the algae do not dry out during the test. If needed, add culture medium and take care that no medium is poured directly onto the surface of the specimen.

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 instead of filter paper 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.

Specimens with thick coatings (e.g. renders) shall be treated analogously. During the growing phase at (23 ± 2) °C the specimens in the Petri dishes shall be illuminated, using light of (1 000 ± 200) lx and a cycle of each 16 h illumination and 8 h darkness.

8.7 Assessment

The algal growth on the treated specimen is assessed in relation to the growth on the untreated specimen at 14 days, 21 days, 28 days and 35 days after the inoculation, using the following scale:

0 no algal growth on the surface of the specimen and in the Petri dish;

1 less algal growth on specimen containing film preservatives compared to non-preserved ones;

2 equal or more algal growth on the specimen containing film preservatives compared to non preserved ones.

The assessment shall be 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 35 days. The testing may be considered complete at an earlier stage provided that the unpreserved specimens have severe algal growth. At the same time the preserved specimens shall be rated. The duration of test selected by the test laboratory for the assessment shall be recorded.

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 algal growth;

— uncoated and sterilized substrates show no algal growth.

NOTE 2 It is considered that for the purpose of this test the efficacy of film preservatives in a coating is demonstrated if the samples containing film preservatives are rated “0” or “1”.

9 Test report

The test report shall include at least the following information:

a) microorganisms used and cell counts 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 15458:2014);

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) light intensity;

j) validity of the test;

k) result of rating of each specimen;

l) any deviation from the test method specified;

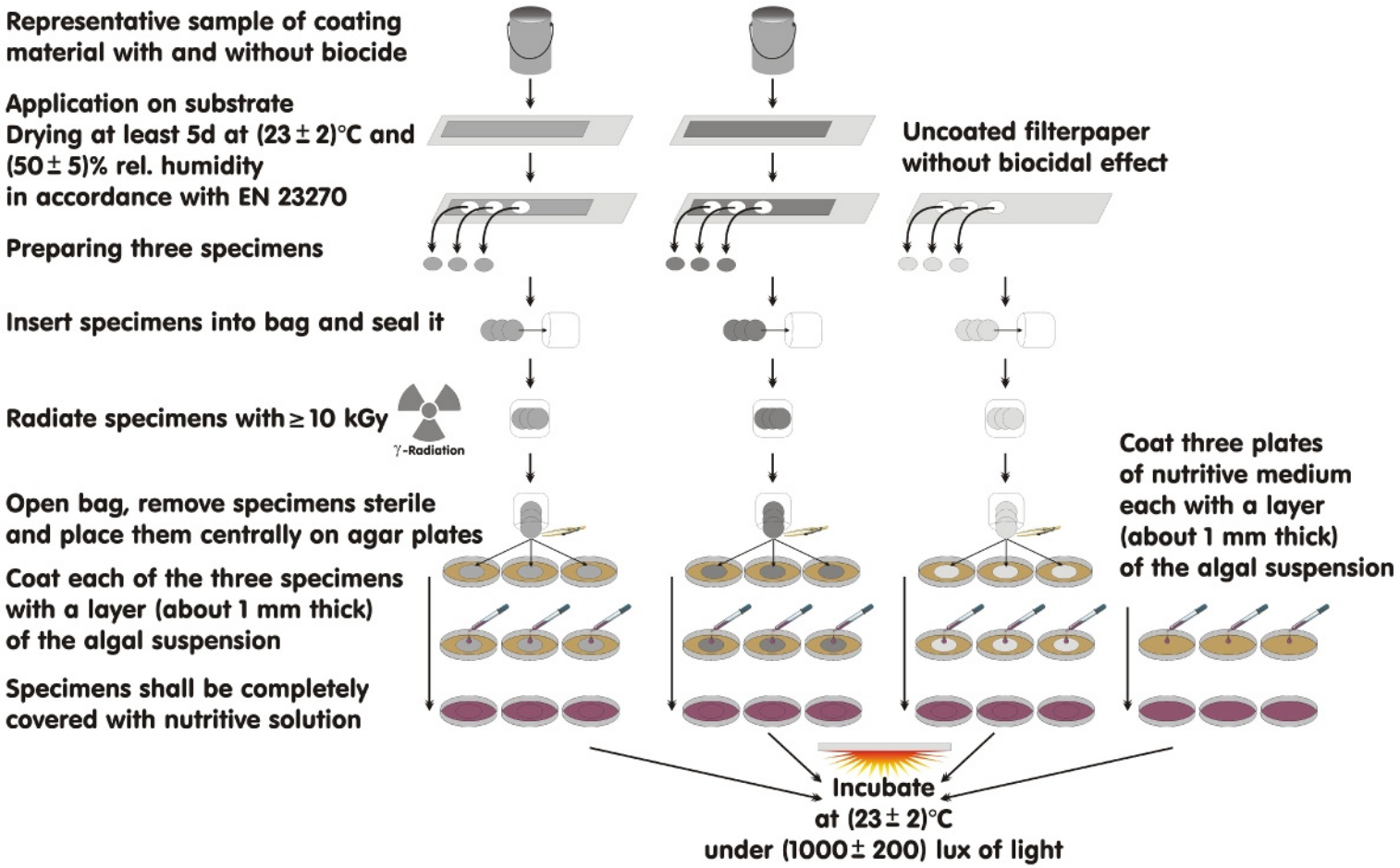
m) any unusual features (anomalies) observed during the test;

n) 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 algae



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

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