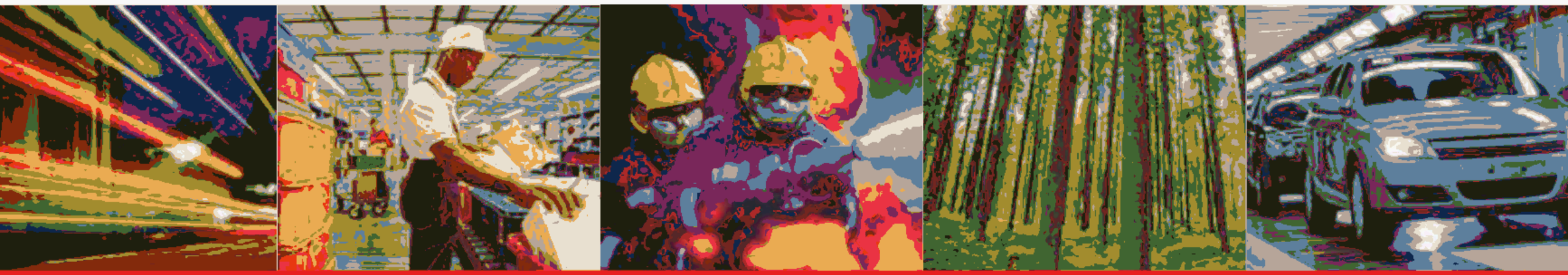
BS EN 15458:2022



BSI Standards Publication

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

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National foreword

This British Standard is the UK implementation of EN 15458:2022. It supersedes BS EN 15458:2014, which is withdrawn.

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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 vérifier 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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European foreword

This document (EN 15458:2022) 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of an identical text or by endorsement, at the latest by September 2022, and conflicting national standards shall be withdrawn at the latest by September 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 15458:2014.

The main changes compared to the previous version are as follows:

a) new terms and definitions have been added;

b) the use of terms and definitions throughout the document corrected;

c) the document has been editorially revised and the normative references have been updated.

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Introduction

This document identifies criteria to assess 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 Product Regulation 528/2012 (Regulation EU No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the placing of biocidal products on the market – BPR).

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

1 Scope

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

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

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

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. 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)*

EN ISO 15528, *Paints, varnishes and raw materials for paints and varnishes - Sampling (ISO 15528)*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

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]

3.2
sample
portion of coating material to be tested

3.3
test sample
strip of filter paper without biocidal effect covered with the coating material to be tested

NOTE See also Figure A.1.

3.4
test specimen
punched-out portion of a test sample

NOTE See also Figure A.1.

4 Principle

To determine 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 test specimen after incubation. The method described in this document is a semiquantitative, comparative method between coatings with and without film preservatives.

5 Apparatus and materials

5.1 Cutting device for preparing the test 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 If applicable, 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's modified Basal medium as specified in the method (see 8.1).

5.10 Bold's Basal modified medium 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 or quantummeter.

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 test specimens

7.1 Sampling

Take a representative sample of the coating material or of the coating system for testing in accordance with EN ISO 15528 and examine and prepare it 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/system to be tested. The application rate recommended by the coating manufacturer for normal use should be employed.

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 test specimens

After conditioning, three test specimens, each of a diameter of 55 mm shall be prepared from the test samples. The test specimens shall be sealed in a plastic or paper 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 test specimens coated with material containing the film preservative, three test specimens coated with the same coating material without film preservative and three test specimens of the uncoated substrate shall be tested.

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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.

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 a) to f) in 8.2;

b) 1 ml each of trace element 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 (121 °C ±2 °C, at least 20 min, at least 1 000 hPa). For the test both solid (with 1,5 % agar) and also liquid nutritive medium are required.

8.2 Preparation of the Bold's Basal Medium stock solutions

Weigh the chemical to the nearest 0,1 g (for trace element stock solutions to the nearest 0,01 g) in a suitable flask and add the specified amount of distilled water. Homogenize the Bold's Basal medium 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		
h)	KOH	31 g	Distilled water	1 000 ml
i)	$FeSO_4 \cdot 7H_2O$ (acidified distilled water = 1 ml concentrated H_2SO_4 in 999 ml distilled water)	4,98 g	Distilled water (acidified)	1 000 ml
j)	H_3BO_3	11,42 g	Distilled water	1 000 ml
k)	$ZnSO_4 \cdot 7H_2O$	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

Pour the algal nutritive agar into sterile Petri dishes after cooling to about 55 °C to 60 °C. To examine the films, the Petri dishes shall be filled with 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.

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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.

8.4 Preparation of stock cultures and sub-cultures

8.4.1 Stock cultures:

Sterile test tubes with agar slant shall be prepared. A volume of 10 ml to 20 ml of Bold's Basal (BB) algal nutritive agar should be poured into test tubes, depending on the size of the test tube. The test tubes containing the BB algal nutritive agar should be closed with a test tube cap, sterilized and stored in an inclined position under sterile condition to allow the agar to solidify." 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 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.

8.4.2 Sub-cultures:

Sub-cultures should be prepared from the stock cultures using conical flasks containing Bold's Basal Medium. The sub-cultures should be incubated as described above.

NOTE Experience shows that these sub-cultures will have grown after 7 days to 14 days, to such an extent that they can be used for subsequent experiments. 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, algal culture shall be mixed with 200 ml of Bold's Basal Medium. 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, combine the required individual algal cultures with Bold's Basal Medium in the in the same ratio as mentioned above (e.g. two different test species will require 2 × 200 ml algal suspension plus 2 × 200 ml Bold's Basal Medium equalling a total volume of 800 ml).

8.6 Inoculation and incubation (see Annex A)

In addition to the coated and uncoated test specimens (see 7.4) a further three Petri dishes containing Bold's Basal agar medium only shall be inoculated.

In a safety cabinet the sterilized test specimens shall be placed centrally onto the Bold's Basal agar medium in the Petri dishes. The coated surface of the test specimen shall be face up and there shall be full contact without air bubbles between the test specimen and the surface of the culturing medium. Cover the test specimens with a layer of the algal suspension (using a sterile pipet tip). The algae in the suspension should be kept evenly distributed by shaking the suspension before application to the test specimen. Ensure that the test specimen is completely covered with the nutritive solution and that the algae do not dry out during the test. If needed, add culturing medium and take care that no medium is poured directly onto the surface of the test 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

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4) cfu = colony forming units.

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 similarly. During the growing phase at (23 ± 2) °C the test 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 test specimen is assessed in relation to the growth on the untreated test 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 test specimen;

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

2 equal or more algal growth on the test specimen containing film preservatives compared to unpreserved test specimens.

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 test specimens are overgrown with algae. At the same time the preserved test specimen should be rated. The duration of the experiment and the time of assessment should be recorded. The test shall be rejected and repeated, if:

— contamination by other microorganisms on the surface of the test specimen occurs;

— test specimens without film preservative 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) the details necessary to identify the product tested;

b) the reference to this document (EN 15458:2022);

c) the microorganisms used and cell counts applied in the test;

d) the active substance(s) and concentration;

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

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

g) the method and extent of conditioning before testing;

h) the test temperature;

i) the light intensity;

j) the validity of the test;

k) the result of rating of each test specimen;

l) any deviation from the test method specified;

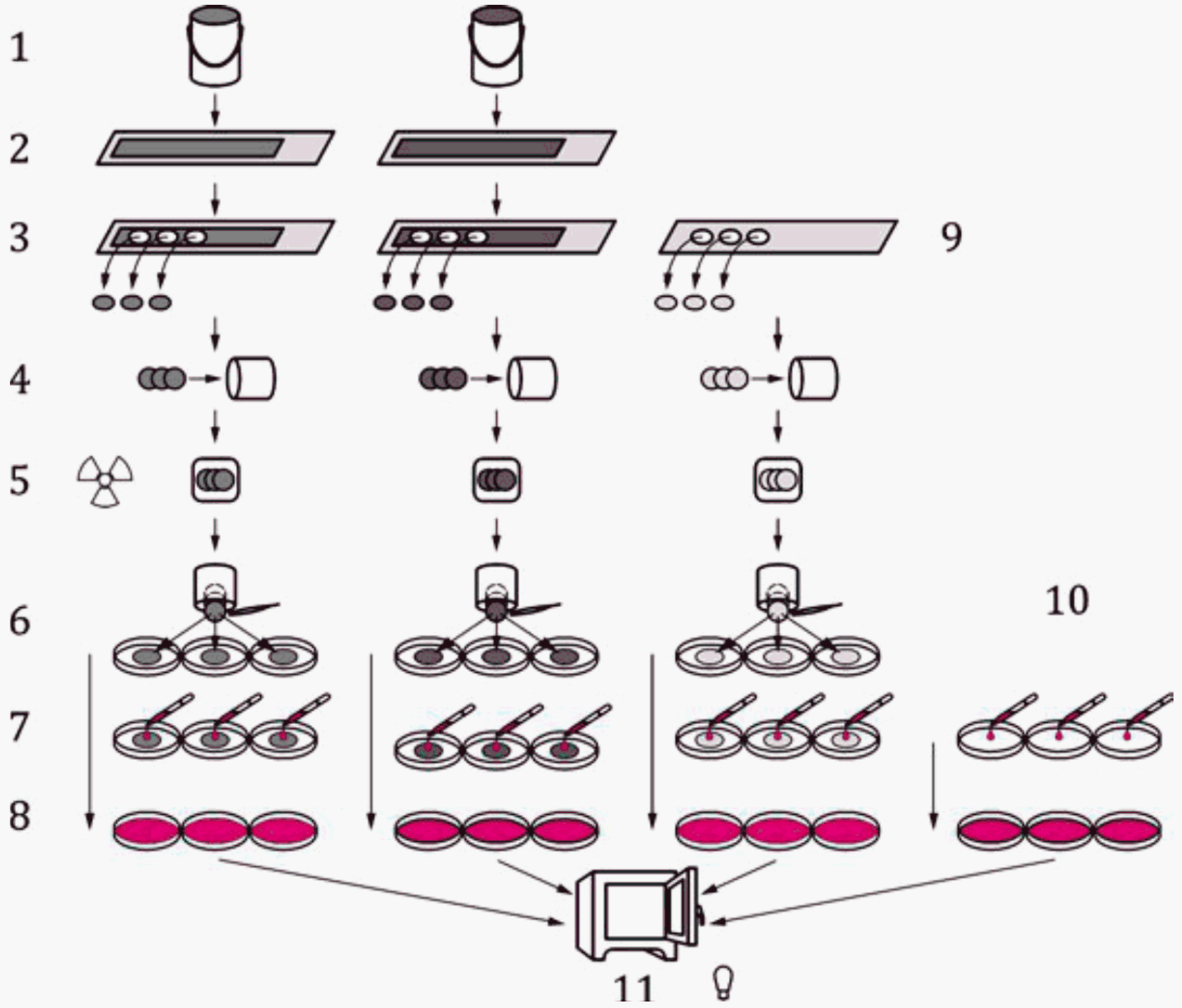
m) any unusual features (anomalies) observed during the test;

n) the 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.

Annex A
(informative)

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



Key

1 representative sample of coating material with and without biocide

2 application on substrate, drying at least 5 days at (23 ± 2) °C and (50 ± 5) % rel. humidity in accordance with EN 23270

3 preparing three specimens

4 insert specimens into bag and seal it

5 radiate specimens with ≥ 10 kGy

6 open bag, remove specimens and place them centrally on MEA plates

7 coat each of the three specimens with a layer (about 1 mm thick) of the algal suspension

8 specimen shall be completely covered with nutritive solution

9 uncoated filter paper without biocidal effect

10 coat three plates of nutritive medium each with a layer (about 1mm thick) of the algal suspension

11 incubate at (23 ± 2) °C, under 1000± 200 lux of light

Figure A.1 — Scheme of laboratory method

Bibliography

Biocidal Product Regulation 528/2012 (Regulation EU No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the placing of biocidal products on the market – BPR

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