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Plastics — Assessment of the effectiveness of fungistatic compounds in plastics formulations

Plastiques — Évaluation de l'efficacité des composés fongistatiques dans les formulations de plastiques

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

International Standard ISO 16869 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 6, *Ageing, chemical and environmental resistance*.

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Introduction

It is a well known phenomenon that plasticizers as well as other ingredients in plastics formulations can be attacked by bacteria, yeasts and fungi, the latter being the most important deteriogens. Microbial attack results in a reduction of the quality of the plastic, causing embrittlement as well as discoloration. This deterioration is of economic importance.

The prevention of fungal attack can be achieved by the incorporation of a fungistatic compound into the formulation. The function of this fungistat is to inhibit the growth of any fungi present on the surface of the plastic product.

The method described in this International Standard determines the effectiveness of fungistatic compounds incorporated into the plastic against the fungi used in the test.

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Plastics — Assessment of the effectiveness of fungistatic compounds in plastics formulations

WARNING — Handling and manipulation of microorganisms that are potentially hazardous requires a high degree of technical competence and may be subject to current national legislation and regulations. Only personnel trained in microbiological techniques should carry out such tests. Codes of practice for disinfection, sterilization and personal hygiene must be strictly observed.

It is recommended that workers consult IEC 60068-2-10:1988, appendix A "Danger to personnel", and ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

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1 Scope

This International Standard specifies a method for determining the effectiveness of fungistatic compounds in protecting susceptible ingredients like plasticizers, stabilizers, etc., in plastics formulations. The method demonstrates whether or not a plastic product is actively protected against fungal attack.

The evaluation is by visual examination.

The test is applicable to any articles made of plastic that are in the form of films or plates no thicker than 10 mm. In addition, porous materials such as plastic foams may be tested provided that they are in the above-mentioned form.

In contrast to ISO 846, the test films are not sprayed with a fungal spore suspension but covered with a layer of test agar containing spores. It has been found that this leads to a better distribution of the spores as well as providing a good supply of water necessary for spore germination on plastic surfaces that are normally hydrophobic.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 291:1997, *Plastics — Standard atmospheres for conditioning and testing*

EN 554:1994, *Sterilization of medical devices — Validation and routine control of sterilization by moist heat*

3 Terms and definitions

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

3.1
plastic susceptible to fungal attack
plastic material that contains in its formulation one or more nutrients that support fungal growth

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3.2
fungistat
a compound that prevents fungal growth on a material that is normally susceptible to fungal attack

4 Principle

Test specimens are exposed to a suspension of mixed fungal spores. The spores are applied to the surface of the test specimen in a thin layer of an agar medium without an added carbon source. In this way, uniform distribution of the spores is achieved as well as an optimum supply of water.

The absence of fungistatic agents in the plastic material will lead to germination of the fungal spores and initial growth. When the ingredients in the material are susceptible to fungal attack and no active fungistat is included in the formulation, further growth and sporulation will occur over and around the test specimen.

The presence of an active fungistat in the material will lead to suppression of spore germination and initial growth in the area over and around the test specimen. Fungistatic agents can migrate into the agar around the test specimen, thereby suppressing germination and appearing to give an increased zone of inhibition.

Although not relevant to the interpretation of the test results, the inhibition zone can be an indication of the behaviour of the fungistat under test.

5 Apparatus and materials

5.1 Apparatus

Sterilize all glassware and all parts of the rest of the apparatus that will come into contact with the culture media and/or reagents (except those which are supplied sterile) by one of the following methods:

method A: autoclave (see 5.1.2) at 121 °C for a minimum of 15 min;

method B: use a dry-heat sterilizer (see 5.1.2) at 180 °C for at least 30 min, at 170 °C for at least 1 h, or at 160 °C for at least 2 h;

method C: use a membrane-filtration system of pore size 0,45 μm.

5.1.1 **Incubator**, maintained at 24 °C ± 1 °C.

5.1.2 **Sterilization apparatus**:

5.1.2.1 For moist-heat sterilization, an **autoclave** complying with the requirements of EN 554.

5.1.2.2 For dry-heat sterilization, a **hot-air oven** maintained at one of the temperatures specified in method B above.

5.1.2.3 For membrane-filtration sterilization, a **membrane-filtration apparatus**, of pore size as specified in method C above.

5.1.3 **Analytical balance**, accurate to ± 0,1 mg.

5.1.4 **Laboratory centrifuge**, speed 2 000 rpm to 5 000 rpm.

5.1.5 **Counting chamber** (for direct counting with the help of a microscope).

5.1.6 **Microscope**, magnification × 100.

5.1.7 **pH-meter**, having an accuracy of ± 0,1 pH-units, capable of temperature correction.

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5.1.8 Vortex shaker, operating at 2 000 rpm to 2 500 rpm.

5.1.9 Containers: test tubes, flasks or bottles of suitable capacity.

5.1.10 Petri dishes, 90 mm to 100 mm in diameter and at least 15 mm deep.

5.1.11 Graduated pipettes, with nominal capacities of 1,0 ml and 15,0 ml. Calibrated automatic pipettes may be used.

5.1.12 Graduated measuring cylinder, minimum capacity 30 ml.

5.1.13 Glass beads, diameter 3 mm to 5 mm.

5.2 Culture media and reagents

All reagents shall be of analytical grade and/or of a grade appropriate for microbiological purposes.

5.2.1 Water

Any water used shall be distilled or deionized and have a conductivity of < 1 µS/cm.

5.2.2 Malt-extract agar (MEA)

Malt extract	30,0 g
Soya peptone	3,0 g
Agar-agar	15,0 g
Water (5.2.1)	to make up to 1 000 ml

Sterilize in the autoclave (see 5.1.2). After sterilization, the pH of the medium shall be 7,0 ± 0,2.

5.2.3 Chaetomium agar

$NaNO_3$	2,0 g
$MgSO_4 \cdot 7H_2O$	0,5 g
KCl	0,5 g
$Fe_2(SO_4)_3 \cdot H_2O$	0,01 g
KH_2PO_4	0,14 g
K_2HPO_4	1,20 g
Agar-agar	15,0 g
Yeast extract	0,02 g
Microcellulose or Carboxymethyl-cellulose (Na salt)	20,0 g 10,0 g
Water (5.2.1)	to make up to 1 000 ml

Sterilize in the autoclave (see 5.1.2). After sterilization, the pH of the medium shall be 7,2 ± 0,2.

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5.2.4 Wetting agent

Prepare a 5 % (*m*/*V*) stock solution of polysorbate 80 (polyoxyethylenesorbitane monooleate) in water. To harvest the spores, dilute the stock solution with water to 0,05 % (*m*/*V*).

5.2.5 Stock solution for nutrient-salt solution and agar

NaCl	0,5 g
$FeSO_4 \cdot 7H_2O$	0,2 g
$ZnSO_4 \cdot 7H_2O$	0,2 g
$MnSO_4 \cdot 1H_2O$	0,06 g
Water (5.2.1)	to make up to 1 000 ml

Before storage for a lengthy period, the stock solution shall be sterilized by membrane filtration.

5.2.6 Nutrient-salt solution

KH_2PO_4	2,62 g
$Na_2HPO_4 \cdot 2H_2O$	0,2 g
$MgSO_4 \cdot 7H_2O$	0,7 g
NH_4NO_3	1,0 g
Stock solution (5.2.5)	10 ml
Water (5.2.1)	to make up to 1 000 ml

Sterilize in the autoclave (see 5.1.2). After sterilization, the pH of the medium shall be 5,5 ± 0,2.

5.2.7 Nutrient-salt agar

KH_2PO_4	2,62 g
$Na_2HPO_4 \cdot 2H_2O$	0,20 g
$MgSO_4 \cdot 7H_2O$	0,70 g
NH_4NO_3	1,0 g
Agar-agar	15,0 g
Stock solution (5.2.5)	10 ml
Water (5.2.1)	to make up to 1 000 ml

Heat the prepared medium till the agar-agar is molten, then measure the temperature and adjust the pH to 5,5 ± 0,1. Sterilize in an autoclave (see 5.1.2).

Agar plates shall be freshly prepared when no validated method for long-term storage (longer than 3 days) is available. The sterilized solutions may be stored for up to 3 months. The storage conditions shall preclude any evaporation occurring.

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5.3 Organisms and cultivation

5.3.1 Test organisms

5.3.1.1	*Aspergillus niger*	ATCC 6275
5.3.1.2	*Chaetomium globosum*	ATCC 6205
5.3.1.3	*Paecilomyces variotii*	CBS 628.66
5.3.1.4	*Penicillium funiculosum*	ATCC 9644
5.3.1.5	*Trichoderma longibrachiatum*	ATCC 13631

The test fungi shall be obtained from a national culture collection (e.g. ATCC = American Type Culture Collection, USA; CBS = Centraalbureau voor Schimmelcultures, NL).

If there are particular reasons for doing so, and by agreement between the interested parties, other fungi may be used. In any case, all strains used shall be listed in the test report.

5.3.2 Culture conditions

Cultivation of strains 5.3.1.1 and 5.3.1.3 to 5.3.1.5 shall be on a malt-extract agar (5.2.2) slant in a test tube at 24 °C ± 1 °C for 14 to 21 days.

Cultivation of strain 5.3.1.2 shall be on Chaetomium agar (5.2.3) at 24 °C ± 1 °C for 14 to 21 days.

Stock cultures may be kept on agar slants or, preferably, freeze-dried or frozen.

6 Test specimens

6.1 Shape and dimensions

Sterilize a suitable punch and punch out round specimens from each test film to obtain discs with a diameter of 1 cm to 3 cm as required. The thickness of the specimens shall not exceed 10 mm.

6.2 Number of specimens

Prepare at least three replicate specimens of each material to be evaluated.

7 Preparation of specimens

7.1 Cleaning

Handling them with tweezers, clean the specimens mechanically (if necessary with a brush) and store in a clean container. Carry out all subsequent handling of the specimens using tweezers to avoid contamination.

7.2 Labelling and storage

Labelling or marking may result in interactions with the plastic during the test. Therefore store the specimens separately in closed containers (e.g. Petri dishes) at ambient temperature. Mark the containers and not the specimens.

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8 Procedure

8.1 Test temperature

Prepare and condition the specimens in an atmosphere complying with ISO 291 Class 2 [23 °C ± 2 °C and (50 ± 10) % rh].

8.2 Filling the Petri dishes

After sterilization, pour 20 ml of the nutrient-salt agar (5.2.7) into each Petri dish, allow to solidify and dry until no water is visible on the surface.

8.3 Arrangement of test specimens

Place the test specimen discs separately, as flat as possible, in the middle of the solidified medium.

If the test specimens are thicker than 5 mm, punch holes in the agar using a punch of the same size as that used when preparing the specimens. The punch shall be sterilized, e.g. by flame treatment. Fit the specimens into the holes in the agar.

8.4 Inoculation of the test specimens

8.4.1 Preparation of the spore suspension

Produce a mixed spore suspension from well sporulated cultures as follows:

Introduce into each culture tube (see 5.3.2) 5 ml of wetting-agent solution (5.2.4). Gently scrape the surface of the sporulating culture with a sterile inoculation needle to obtain an aqueous suspension of the spores. Gently shake the culture tube to disperse the spores. Pour the liquid into a sterile Erlenmeyer flask containing 10 to 20 glass beads. Repeat this procedure with the same culture tube and the same Erlenmeyer flask three times. Then shake the spore suspension of each fungal culture with the glass beads and filter through a thin layer of sterile cotton or glass wool to remove any mycelial fragments.

Determine the density of each spore suspension in a counting chamber. The spore count shall be at least 5×10^6 spores/ml.

Mix together equal volumes of each spore suspension immediately before use with a shaker (5.1.8). Individual spore suspensions may be stored for up to 4 days at 4 °C, for up to 2 months at – 18 °C or for up to 12 months at – 196 °C.

8.4.2 Inoculation of the nutrient-salt overlay agar

For each specimen, melt 15 ml of nutrient-salt agar (5.2.7) in a tube and maintain at 45 °C to 48 °C in a water bath until required. Inoculate each tube with 1 ml of the mixed spore suspension (see 8.4.1) immediately before use.

8.4.3 Overlay of specimen

Pour the inoculated molten nutrient-salt agar onto the surface of the base agar and test specimen to form a thin second layer. Rotate the Petri dish carefully to obtain an even layer.

8.4.4 Incubation

Incubate the inoculated Petri dishes at 24 °C ± 1 °C and no less than 85 % rh for 21 days.

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