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PLASTICS

RECOMMENDED PRACTICE FOR THE EVALUATION OF THE RESISTANCE OF PLASTICS TO FUNGI BY VISUAL EXAMINATION

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BRIEF HISTORY

The ISO Recommendation R 846, *Plastics – Recommended practice for the evaluation of the resistance of plastics to fungi by visual examination,* was drawn up by Technical Committee ISO/TC 61, *Plastics,* the Secretariat of which is held by the United States of America Standards Institute (USASI).

Work on this question by the Technical Committee began in 1957 and led, in 1964, to the adoption of a Draft ISO Recommendation.

In April 1965, this Draft ISO Recommendation (No. 813) was circulated to all the ISO Member Bodies for enquiry. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

Argentina	Greece	Poland
Australia	Hungary	Portugal
Austria	India	Romania
Belgium	Israel	Switzerland
Brazil	Italy	U.A.R.
Canada	Japan	United Kingdom
Czechoslovakia	Netherlands	U.S.A.
France	New Zealand	U.S.S.R.

One Member Body opposed the approval of the Draft :

Sweden

The Draft ISO Recommendation was then submitted by correspondence to the ISO Council, which decided, in October 1968, to accept it as an ISO RECOMMENDATION.

ISO Recommendation R 846 October 1968

PLASTICS

RECOMMENDED PRACTICE FOR THE EVALUATION OF THE RESISTANCE OF PLASTICS TO FUNGI BY VISUAL EXAMINATION

INTRODUCTION

Micro-organisms vegetate on plastics containing nutritive substances, necessary for their growth, which are either contained in the plastics as components or are deposited subsequently thereon either in processing or in use.

Thus, on material which is itself fungus-resistant, fungi can vegetate only in the presence of impurities. In this case, the fungus may affect the plastics by its metabolic products influencing indirectly the properties of the plastics.

1. SCOPE

1.1 This ISO Recommendation describes the procedure for the evaluation of the resistance of plastics to attack by fungi when inoculated by these organisms.

This procedure provides for visual observation as to whether or not plastics are subject to fungal growth but it does not provide data about changes of chemical and physical properties caused by micro-organisms.

1.2 According to the end application of a material, certain requirements, such as water resistance, resistance to washing, resistance to weathering, or heat stability may have necessitated certain treatments. Therefore it should be stated explicitly in what condition the test material is being tested, i.e., its method of pre-treatment should be indicated.

NOTE. – Since the procedure involves handling and working with fungi, it is recommended that personnel trained in microbiology should perform the part of the procedure involving handling of organisms and inoculated specimens.

1.3 Two methods of test are described in this ISO Recommendation : *Method A* and *Method B*.

1.3.1 *Method A* determines whether the plastics under test are inert or serve as a nutritive medium for the growth of micro-organisms. The resistance of a material is judged visually according to the degree of fungal growth on its surface.

1.3.2 *Method B* determines the degree of fungal growth on the surface of the material when it is enriched by a nutritive medium. The evaluation is made visually. This method permits the determination of the fungitoxic properties of plastics.

1.4 If the conditions of use in service of the material are taken into consideration, Method A may be considered sufficient; if not, the test is then continued according to Method B.

1.5 For example, if plastics are used under conditions which exclude surface contamination by organic substances, Method A is sufficient. However, materials used under conditions involving strong surface contamination should be tested according to Method B.

1.6 In order to save time, it is recommended that the two test Methods (A and B) should be started simultaneously.

2. METHOD A

2.1 Scope

Method A is intended to determine the ability of plastics themselves to support the growth of fungi, i.e. to serve as a nutritive medium.

For this reason, the test specimens are placed on a mineral-salts agar medium (2.3.1.3) and inoculated with the spore suspension of the fungi in an aqueous mineral-salts solution (2.3.1.4) in order to provide for conditions under which the plastics are a nutritive medium for fungi.

2.2 Test specimens

2.2.1 The size of the test specimens is optional, but not less than three replicate test specimens should be used.

NOTE. – Recommended dimensions of the test specimens are as follows :

30 to 50 mm square pieces, with a maximum thickness of 4 mm.

2.2.2 The specimens are tested under optimum conditions for fungal growth, i.e., at 30 ± 2 °C and at 95 to 100 % relative humidity.

2.2.3 The specimens are tested as delivered, without cleaning.

NOTE. – In order to make sure that secondary contamination with nutrient substances does not interfere with the test, the specimens may be cleaned according to section A.5 of the Annex. When cleaned test specimens are used, this fact should be mentioned in the test report, under section 4.

2.3 Preparation of fungal species for test

2.3.1 *Nutritive media.* The following nutritive media are suitable for the culturing of fungi.

2.3.1.1 *Modified Czapek-Dox agar, containing saccharose.*

$NaNO_3$	2	g
KH_2PO_4	0.7	g
K_2HPO_4	0.3	g
KCl	0.5	g
$MgSO_4 . 7 H_2O$	0.5	g
$FeSO_4 . 7 H_2O$	0.01	g
Saccharose	30	g
Distilled water	1000	ml
Agar	20	g

2.3.1.2 *Modified Czapek-Dox agar, containing cellulose.* In the nutritive medium mentioned in clause 2.3.1.1 above, saccharose is replaced by cellulose in the form of strips of filter paper.

NOTE. – On modified Czapek-Dox agar containing saccharose the fungal strains No. 1, 2, 3 and 4 can be cultured. Modified Czapek-Dox agar containing cellulose can be used for culturing the strains No. 2, 4 and 5 given below (see clause 2.3.2).

2.3.1.3 *Mineral-salts agar.* This is identical with the composition of modified Czapek-Dox agar (2.3.1.1) but without the saccharose.

2.3.1.4 *Aqueous mineral-salts solution.* This is identical with the composition of modified Czapek-Dox agar (2.3.1.1), but without the saccharose and agar.

NOTE. – For the preparation of nutritive media, see the Annex.

2.3.2 *Test Fungi.* The inoculation of specimens is carried out by applying a mixture of aqueous spore suspensions of the following strains of fungi :

(1)	*Aspergillus niger*	v. Tieghem
(2)	*Penicillium funiculosum*	Thom
(3)	*Paecilomyces varioti*	Bainier
(4)	*Trichoderma viride*	Pers ex Fr
(5)	*Chaetomium globosum*	Kunze

NOTE. – Besides the above-mentioned five strains of fungi, additional species may be used. If additional species are used, they should be mentioned in the test report. The test fungi used in preparing the cultures should in all cases be well defined species obtained from official biological centres, the addresses of which can be obtained from National Standards Associations in each country.

2.3.3 *Preparation of the spore suspension.* To the well sporulated fungal cultures, which have been maintained at 30 °C for 2 to 4 weeks, add about 5 to 7 ml of sterilized distilled water containing 0.01 % of a suitable wetting agent.* The surface is then gently rubbed with the inoculation needle for the purpose of transferring the spores into the aqueous phase. By gently shaking the culture tube, the spores are liberated from the fruiting bodies. This is repeated with each culture. The shaken spore suspension of each culture is filtered through a thin layer of sterile cotton or glass wool in order to remove mycelial fragments and other debris, into the same sterile flask (see Note below). The filtered mixed spore suspension is centrifuged aseptically and the supernatant liquid is discarded. The residue is re-suspended in about 50 ml of sterile water and centrifuged. This procedure is repeated three times. The final washed residue is suspended in 100 ml of sterile aqueous mineral-salts solution (2.3.1.4).

NOTE. – It may be helpful to work with a known number of spores by counting and diluting, for example, by separately filtering each spore suspension, counting with the help of a counting chamber of a nephelometer and diluting with sterilized distilled water to a concentration of 1 to 2 million spores per millilitre. After mixing, the spore suspensions are cleaned as described above.

2.4 Inoculation of specimens

Sufficient mineral-salts agar (2.3.1.3) is poured into suitable sterile Petri dishes to provide a solidified agar layer from 3 to 4 mm depth. After the agar has solidified, the test specimens are placed on the surface of the agar (see Note below). The surface, including the surface of the test specimens, is inoculated by spraying (if preferred) with the spore suspension obtained according to clause 2.3.3 and the Petri dishes are then covered and put in an incubator.

However, to avoid any possible dispersal of spores in the air, the test specimens are sprayed in suitable boxes. When the spore suspension is sprayed from a flask or a sprayer, it is necessary to shake it from time to time in order to keep the spores in proper suspension.

The spore suspension should be used for inoculation of test specimens within 8 hours after its preparation.

For a control determination of spore growth, two Petri dishes with the complete nutritive medium (modified Czapek-Dox agar (2.3.1.1) or (2.3.1.2) depending on the species) are sprayed by the same spore suspension and cultured together with the specimens. If during 3 to 4 days no spore growth in the control Petri dishes is observed, the test is repeated with new specimens and a fresh suspension.

NOTE. – Covered Petri dishes containing mineral-salts agar are considered to maintain the desired humidity for 4 weeks. Covers on large dishes may be sealed with masking tape.

* N-methyltauride, sodium lauryl sulphonate or polyglycol ether.

2.5 Duration of the test

The duration of the test is initially fixed at 28 days. In special cases the test can be extended to any desired length of time.

NOTE. – If the fungal growth is clearly visible to a naked eye before 28 days have elapsed, the test may be considered completed.

2.6 Examination of the growth of fungi on test specimens

After 28 days, the test specimens are removed from the incubator and are inspected for fungal growth. The examination is visual, by the naked eye or, if necessary, with the help of a microscope (magnification 50 or 60 times).

The intensity of fungal growth is rated as follows :

0 – No growth is apparent under the microscope.

1 – Fungal growth is hardly visible to the naked eye, but it is quite apparent under the microscope.

NOTE. – If a microscopic growth is so observed under the microscope, the test specimens are incubated for a further 28 to 56 days.

2 – The growth on the surface is visible to the naked eye but does not cover more than 25 % of the test surface.

3 – The growth on the surface is visible to the naked eye and covers more than 25 % of the test surface.

2.7 Evaluation of test results

The evaluation of the resistance of plastics to the growth of fungus is determined according to the degree of fungal growth. Table 1 gives the evaluation of resistance of plastics.

TABLE 1

Intensity of fungal growth	Evaluation of testing material
0	Plastics are not nutritive media for micro-organisms (they are inert or fungitoxic).
1	Plastics contain nutritive substances or are contaminated in such a small degree that they permit only a slight growth.
2 , 3	Plastics are not resistant to a fungal attack and contain nutritive substances suitable for the development of micro-organisms.

The visual observations on each individual test specimen should be recorded. If the visual observations on replicate test specimens vary over a range of two scale ratings or more, it is recommended that the test should be repeated, using at least six replicate test specimens.

3. METHOD B

3.1 Scope

Method B is intended to determine fungitoxic properties of plastics. It can be applied to those materials which were evaluated by Method A as being inert or showing only slight growth of micro-organisms.

3.2 Test specimens

3.2.1 For this method, the same number of similar test specimens is used, as for Method A (see clause 2.2.1).

3.2.2 Tests made according to Method B are carried out under the same conditions as tests made according to Method A (see clause 2.2.2).

3.2.3 The specimens are tested as delivered, i.e., without further cleaning.

3.3 Preparation of spore suspension

The spore suspension is prepared in the nutritive solution Czapek-Dox (see clause 2.3.1), containing 0.01 % of a wetting agent, without agar, in the same concentration as by Method A.

The spore suspension is filtered through sterile glass wool or cotton. The suspension is made and used in the same quantity as that for Method A (see clause 2.3.3).

3.4 Inoculation of specimens

Specimens are placed on agar plates consisting of modified Czapek-Dox agar containing saccharose (2.3.1.1) and are sprayed with the above spore suspension in the same way as in Method A (see clause 2.4).

For long period tests, in order to maintain the fungal growth on the nutritive substance, the specimens are regularly sprayed with nutritive solution (Czapek-Dox) without spores once in 28 days, the first time after 28 days.

For a control determination of spore growth, two Petri dishes with nutritive medium (modified Czapek-Dox agar (2.3.1.1) or (2.3.1.2) depending on the species) are sprayed by the same spore suspension and placed together with the dishes containing the specimens.

If no spore growth develops in the control Petri dishes during 2 to 4 days, the test is repeated with new specimens and a fresh suspension.

3.5 Duration of the test

The duration of the test is initially fixed at 28 days. In special cases, the test can be extended to any desired length of time.

3.6 Examination of the growth of fungi on test specimens

Upon completion of the test, the test specimens are removed from the incubator and are examined by the naked eye or with the help of a microscope (magnification 50 or 60 times). According to the observation of the fungal growth on the surface of the test specimen, the following degrees of growth can be determined :

0 – No fungal growth is apparent even under the microscope. Inhibition zone absent or present; if present, the dimension in millimetres should be stated.

1 – Fungal growth is hardly visible to the naked eye, but it is quite apparent under the microscope.

NOTE. – If a microscopic growth is observed under the microscope, the test specimens are incubated for a further 28 up to 56 days.

2 – Slight growth covering less than 25 % of the test surface.

3 – Medium growth covering 25 to 50 % of the test surface.

4 – Considerable growth covering 50 % or more of the test surface.

5 – Heavy growth covering the entire test surface.

3.7 Evaluation of test results

TABLE 2

Intensity of fungal growth	Evaluation of testing material
0	Strong fungitoxic effect.
0 + zone of inhibition	Strong fungitoxic effect due to diffusing substance.
1	Not a quite complete fungitoxic effect.
2 to 5	Decreasing effectiveness to complete absence of fungitoxic effect.

The visual observations on each individual test specimen should be recorded. If the visual observations on replicate test specimens vary over a range of two scale ratings or more, it is recommended that the test should be repeated, using at least six replicate test specimens.

4. TEST REPORT

The test report should include the following :

(*a*) Specification of the material and its pre-treatment.

(*b*) Specification of the applied fungal species.

(*c*) Specification of the test method (A or B).

(*d*) Method of application (cleaning of the specimen).

(*e*) Duration of test.

(*f*) Test results.

(*g*) Special observations regarding changes of the specimen, i.e., discolouration, cloudiness, infection by bacteria or fungi other than test organisms, etc., characteristics of growth, (i.e., dominating species, influence on the fructification, etc.)

ANNEX

A.1 Preparation of the modified Czapek-Dox agar containing saccharose.

In order to prevent precipitation the following procedure is recommended.

The chemicals, with the exception of the phosphates, are weighed and poured into an Erlenmeyer flask of 2 litre content. After the addition of 800 ml distilled water, the flask is mildly heated to dissolve the chemicals. The 20 g agar is added, the nutritive medium is left standing for some hours and then it is heated until the dissolution of agar is complete. The phosphates are weighed and separately dissolved in 200 ml of distilled water in an Erlenmeyer flask of 500 ml. Both solutions are then sterilized for 20 to 30 minutes at 120 °C. After sterilization, the two solutions are mixed under sterile conditions.

A.2 Preparation of the modified Czapek-Dox agar containing cellulose.

The mixture is prepared as prescribed above, without adding any saccharose. Addition of cellulose is described in the Note of section A.3 below.

A.3 Filling nutritive medium into the test tubes.

The warmed liquid nutritive medium is filled into the test tubes by a funnel extended with rubber tubing (about 7 cm long), which is closed by a tapered glass-tube. A spring clip in the middle of the tubing serves to regulate the flow of the nutritive medium into the test tubes. The rubber should not contain any fungitoxic ingredient.

The test tubes (size 160 mm X 16 mm) are filled with 7 ml of nutritive medium each, then stoppered with cotton stoppers and sterilized for 30 minutes in an autoclave at 120 °C. After sterilization, they are instantly so laid down that the upper end is resting upon a 1 cm thick rod. The nutritive medium spreads, forming a surface the narrower end of which is directed to the tube neck, but it should not touch the stopper in order to avoid soaking, which could cause infection from the outside.

The slanted nutritive medium containing saccharose is allowed to solidify and is then inoculated with the individual strains of fungi.

NOTE. – For the preparation of the modified Czapek-Dox agar containing cellulose, the following procedure is recommended. Test tubes (size 160 mm x 16 mm) are filled with 7 ml of mineral-salts agar (section 2.3.1.3) and sterilized according to the procedure described in section A.3 above.

The slanted incomplete mineral salts agar is allowed to solidify. Strips of filter paper * (70 mm x 10 mm) are sterilized for 20 to 30 minutes at 120 °C and gently laid down on the surface of the solid medium. Then the complete medium is inoculated with the individual strains of fungi.

A.4 Maintaining the stock of cultures of test fungi.

To keep the cultures in normal condition, it is necessary to sub-culture each species periodically, at least every 4 weeks. The recommended procedure is the following :

The handling is carried out near the flame of a bunsen burner. The test tubes containing the sterile nutritive medium and the old culture are taken by the left hand. The stoppered test tube ends are pointing towards the flame. In the right hand is held the inoculation needle which is thoroughly sterilized by the flame. Then, without putting down the inoculation needle, the cotton stopper is removed from the test tube. With the help of the inoculation needle some of the spore from the culture is taken and transferred to the surface of the sterile nutritive medium by gently rubbing it. The outside of the stopper ends is sterilized above the flame and put back into the test tubes (see Note below).

The inoculated test tubes are deposited in a thermostat and cultured at a temperature of 30 °C for a period of 7 days, and then stored at a lower temperature (ambient temperature).

NOTE. – The flaming of cotton stoppers may be hazardous.

* Quality Whatman 3 MM can be used.