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BASIC SAFETY PUBLICATION

PUBLICATION FONDAMENTALE DE SÉCURITÉ

**Environmental testing –
Part 2-10: Tests – Test J and guidance: Mould growth**

**Essais d'environnement –
Partie 2-10: Essais – Essai J et guide: Moisissures**

IEC 60068-2-10:2005

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IEC Central Office
3, rue de Varembe
CH-1211 Geneva 20
Switzerland

Tel.: +41 22 919 02 11
info@iec.ch
www.iec.ch

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INTERNATIONAL ELECTROTECHNICAL COMMISSION

ENVIRONMENTAL TESTING –

Part 2-10: Tests – Test J and guidance: Mould growth

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This consolidated version of the official IEC Standard and its amendment has been prepared for user convenience.

IEC 60068-2-10 edition 6.1 contains the sixth edition (2005-06) [documents 104/365/FDIS and 104/373/RVD] and its amendment 1 (2018-04) [documents 104/740/CDV and 104/790/RVC].

In this Redline version, a vertical line in the margin shows where the technical content is modified by amendment 1. Additions are in green text, deletions are in strikethrough red text. A separate Final version with all changes accepted is available in this publication.

International Standard IEC 60068-2-10 has been prepared by IEC technical committee 104: Environmental conditions, classification and methods of test.

This sixth edition constitutes a technical revision.

The main changes with respect to the previous edition are listed below:

- Two test fungi replaced by two others
- Concentration of the spores defined for each test fungus
- Spores suspension in mineral salt solution additionally introduced
- Pre-conditioning of the specimens by damp heat storage prescribed
- Supersonic aerosolization of the spores suspension as the preferred inoculation method introduced
- Duration of incubation reduced from 84 days to 56 days
- Extent of mould growth grade 2 split into grade 2a and grade 2b
- Detailed information on methods of inoculation given in Annex B
- Annex E: flow-chart deleted

This publication has been drafted in accordance with the ISO/IEC Directives, Part 2.

It has the status of a basic safety publication in accordance with IEC Guide 104.

This standard forms Part 2-10 of IEC 60068 which consists of the following major parts, under the general title *Environmental testing*:

Part 1: General and guidance

Part 2: Tests

Part 3: Supporting documentation and guidance

Part 4: Information for specification writers

Part 5: Guide to drafting of test methods

The committee has decided that the contents of the base publication and its amendment will remain unchanged until the stability date indicated on the IEC web site under "http://webstore.iec.ch" in the data related to the specific publication. At this date, the publication will be

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ENVIRONMENTAL TESTING –

Part 2-10: Tests – Test J and guidance: Mould growth

1 Scope

This part of IEC 60068 provides a test method for determining the extent to which electrotechnical products support mould growth and how any mould growth may affect the performance and other relevant properties of the product.

Since mould growth conditions include high relative humidity, the test is applicable to electrotechnical products intended for transportation, storage and use under humid conditions over a period of some days at least.

2 Normative references

The following referenced documents are indispensable for the application 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.

ISO/IEC 17025:1999, *General requirements for the competence of testing and calibration laboratories*

ISO 846:1997, *Plastics – Evaluation of the action of microorganisms*

MIL-STD-810 F:2000, *Method 508.5 Fungus*

Laboratory Biosafety Manual 2nd Ed., WHO 1993, ISBN 92 4 1544503

3 General description

~~This test covers the inoculation of electrotechnical products with a selection of mould spores followed by a period of incubation under conditions which promote spore germination and the growth of mould.~~

~~Two variations of the test are given. Variant 1 specifies inoculation of the specimen with the mould spores without nutrients whereas variant 2 specifies the inoculation with the mould spores suspended in a nutritive solution which supports mould growth.~~

~~It is advisable to use testing procedures such as specified for plastics in ISO 846 to assess the vulnerability to damage by mould growth of the constructional materials used.~~

~~NOTE—Laboratories for microbiological testing of technical products should be accredited in accordance with ISO/IEC 17025. See further Annex F.~~

3.1 Background

Under certain climatic and environmental conditions, micro-organisms may settle on and colonize the surface of electrotechnical equipment. Their presence or their metabolic products may not only damage the equipment itself, but may also affect the equipment's operability and serviceability. The actions of micro-organisms on equipment are influenced by two different processes: direct action in which the deterioration of material serve as a nutritive substance

for the growth of the micro-organisms and indirect action in which the metabolic products of the micro-organisms generate deterioration.

The preferred method for controlling the effects of micro-organisms is by the selection of materials that do not promote growth. Also acceptable is the treatment, or hermetic sealing, of potentially vulnerable materials and components. Additionally, equipment may not need to be evaluated if it is stored and/or operated throughout its entire life, in conditions unlikely to encourage the growth of micro-organisms. Only if these cannot be achieved is it usually necessary to demonstrate the resistance of complete or partial equipment by testing.

The test procedures and severities of this document are most commonly used to evaluate the resistance of complete or partial equipment, to the damaging effects due to the presence of micro-organisms and their metabolic products. Testing of entire equipment is usually necessary if it is critical that performance be demonstrated after exposure to adverse temperature/humidity conditions that would support the growth of micro-organisms.

An alternative approach which is sometimes used is to consider only the individual materials of which an equipment is composed. This alternative approach may be particularly relevant when the primary concern is with deterioration of structural materials of the equipment rather than its operability and serviceability. In such cases, individual materials may need to be evaluated, only if previous evidence exists as to its resistance to the effects of growth of micro-organisms. The testing procedures in ISO 846 are essentially the equivalent of those set out in this document but applied to specimens comprising samples of material.

Some materials can, when buried in natural soil that has a water holding capacity, exhibit significant degradation in structural characteristics. The evaluation of such conditions are not included in this document. However, should the evaluation of material be required, Method D (soil-burial test) in ISO 846 is suggested. Similarly, if it is necessary to evaluate a material's resistance to biological growth, Method C (resistance to bacteria) in ISO 846 is suggested.

3.2 Selection of test procedure

The test procedures of this document involves exposing electrotechnical products to the action of a selection of test strains of mould spores for a period of incubation under conditions which promote spore germination and the growth of mould. At the end of the exposure, the specimens are assessed for deterioration by visual examination and, if applicable, for any change in mass or other physical properties.

This document contains two basic test procedures Variant 1 and Variant 2:

- a) In Variant 1, specimens are inoculated with a mixed suspension of mould spores in the presence of an incomplete nutritive medium (without a carbon source). The mould can only grow at the expense of the specimen. If the specimens contain no nutritive component, the fungi cannot develop mycelia and there is no deterioration of the material.
- b) In Variant 2, specimens are inoculated with a mixed suspension of mould spores in a (complete) nutritive solution, i.e. with a carbon source. Even if the specimen does not contain any nutritive elements, the mould can grow over the specimen and their metabolic products can attack the material. Any inhibition of the growth on the specimen shows fungal activity of the material or the presence of a fungicidal treatment.

3.3 Considerations when specifying test procedures

Surface contamination in the form of dusts, ~~splashes~~ liquids, condensed volatile nutrients or grease may be deposited upon assembled specimens. This can be brought about by storage and use or transport with the product exposed to the atmosphere or handled without protective covering. This surface contamination can cause an increased colonization by fungi and may lead to greater growth and damage. An assessment of the effect of such contamination can be given by the application of test variant 2.

Due to the difficulty of maintaining the necessary conditions in a very large chamber, ~~a large composite~~ equipment ~~will normally~~ may be tested as a number of sub-units. This will in any case minimize the cost of the test since several sub-units may be so similar in construction that only one of them needs to be tested.

Due to the difficulty of maintaining the necessary conditions in a very large chamber, large equipment may be tested as a number of sub-units. This will in any case minimize the cost of the test since several sub-units may be so similar in construction that only one of them needs to be tested.

The incubation period for determining degradation resistance of equipment is a pragmatic duration which is normally sufficient for the degradation actions of micro-organisms to become apparent. It is not necessarily related to, nor is it intended to replicate, the exposure duration of equipment to adverse temperature/humidity conditions that would support the growth of micro-organisms.

Regardless of the test variant used, specimens are inoculated with a suspension of mould spores typically by spraying. The preferred approach is by means of a supersonic aerosol apparatus, such as that used for therapeutic treatment by inhalation. Such an approach allows a homogeneous distribution of the spores to be achieved on the surfaces of the specimen and consequently results in a high reproducibility of the test results. However, if spraying is not suitable due to the size, design or other properties of the specimen, inoculation with spore suspension by dipping or painting may be carried out, as stated in the relevant specification.

This document contains guidance on the post-test visual inspection of specimens as well as an approach for grading the extent of mould growth. If the purpose of the test is to establish degradation of the operability of electrotechnical equipment, additional electrical and/or mechanical checks will need to be specified by the relevant specification. In such cases, it may be essential that the incubation conditions of temperature and relative humidity surrounding the specimen are maintained throughout such electrical and/or mechanical checks. Additionally, controlled recovery conditions may be needed in order to prevent moisture being absorbed or lost by the specimen before undertaking any required post-test examinations. IEC 60068-1:2013, 4.4.2 indicates an approach that may be used if the specimen needs to be subjected to controlled recovery conditions.

4 Health hazards to operators

This test procedure requires the use of viable mould spores and the application of ambient conditions which promote mould growth.

Therefore before any attempt is made to handle mould cultures, or to carry out steps of the test subsequently described, it is important that the annexes of this standard be studied.

| | |
|---------|--------------------------------|
| Annex A | Danger to personnel |
| Annex B | Inoculation methods |
| Annex C | Recommended safety precautions |
| Annex D | Decontamination procedures |

Laboratory Biosafety Manual, 2nd Ed., World Health Organization 1993, ISBN 92 4 1544503 includes general background reading on safety in facilities dealing with fungi.

5 Description of the test variants

5.1 Test variant 1

After a 28 days incubation period determine

- the extent of mould growth by visual inspection;
- the physical damage caused by mould growth;
- in the case of mould growth the effect on functioning and/or electrical properties if required in the relevant specification.

The incubation period shall be extended to a total of 56 days before checking the function and/or measuring electrical properties if required in the relevant specification.

5.2 Test variant 2

After a simulated contamination with nutrients followed by a 28 days incubation period determine

- the extent of mould growth by visual inspection;
- the physical damage caused by mould growth;
- the effect of the mould growth on functioning and/or electrical properties if required in the relevant specification.

The surface resistance of the specimen will be reduced by application of nutrients for simulation of contamination without any mould growth. This effect should be considered if checking the function and/or measuring electrical properties.

Due to the application of nutrients, mould growth will exist; failing this, a fungicidal effect shall be considered.

6 Reagents and materials

[IEC 60068-2-10:2005](https://standards.iteh.ai/catalog/standards/iec/32418860-03ac-4b9c-8d06-07e5c02b2d44/iec-60068-2-10-2005)

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6.1 Cultures or spores – Supply and conditions

The following fungi shall be used for performing the test (see Table 1). The nature of the attack to be expected from each fungus is indicated for guidance. The spores of all cultures, however, shall be used together in a mixed suspension whatever the nature of the specimen.

The cultures or freeze-dried spores shall be obtained from a recognized mycological cultures collection. They shall be supplied in containers with the date of inoculation of the culture thereon.

A certificate shall confirm the accordance of the culture with the fungus and strain number as specified in Table 1 and/or Annex E.

Cultures and freeze-dried spores shall be handled and stored in accordance with the recommendations of the supplier and the relevant requirements of this standard. Preparing a culture by the test laboratory from a stock culture or from freeze-dried spores the date of inoculation shall be marked on the culture tube.

Table 1 – Test fungi

| No. | Name | Strain No. ³⁾ | Attacks | Note |
|---|-----------------------------------|--------------------------|------------------------------------|-------|
| 1 | <i>Aspergillus niger</i> | ATCC 6275 | many materials | 1) 2) |
| 2 | <i>Aspergillus terreus</i> | ATCC 10690 | plastic materials | 1) 2) |
| 3 | <i>Chaetomium globosum</i> | ATCC 6205 | cellulose | 1) 2) |
| 4 | <i>Hormoconis resinae</i> | DSM 1203 | hydrocarbon based lubricants | – |
| 5 | <i>Paecilomyces variotii</i> | ATCC 18502 | plastics and leather | 1) 2) |
| 6 | <i>Penicillium funiculosum</i> | ATCC 36839 | many materials especially textiles | 1) 2) |
| 7 | <i>Scopulariopsis brevicaulis</i> | ATCC 36840 | rubber | 1) 2) |
| 8 | <i>Trichoderma virens</i> | ATCC 9645 | cellulose, textiles and plastics | 2) |
| 1) Also specified in ISO 846. 2) Also specified in MIL–STD–810 F, Table 508.5–I. 3) See also Annex E. | | | | |

Cultures shall be used for preparing the test spore suspension when they are well sporulated.

This is reached in most cases after a 7 to 14 days incubation period at $(29 \pm 1) ^\circ\text{C}$.

NOTE The supplier of cultures or freeze-dried spores may recommend other conditions to develop the culture.

If the cultures are not for immediate use, they shall be stored in a refrigerator at a temperature between $5 ^\circ\text{C}$ and $10 ^\circ\text{C}$, for a continuous period of not more than six weeks commencing not earlier than 14 days and not later than 28 days from the date of inoculation given on the container.

The lid of the container shall not be opened once the preparation of the spore suspension has started. Only one suspension shall be made from the opened container.

6.2 Preparation of spore suspension

6.2.1 General

The suspension is first prepared in sterilized distilled water, to which has been added a wetting agent with a concentration between 0,005 % and 0,01 %. An agent based on N-methyl-taurine or on dioctyl-sodium sulphosuccinate has been found to be suitable. The wetting agent shall not contain substances which support or inhibit mould growth.

10 ml of the water containing the wetting agent shall be added gently to each culture. A platinum or nichrome wire shall be sterilized by heating to red heat in a flame and allowed to cool. This wire shall be used to gently scrape the surface of the culture to liberate spores.

The liquid shall be slightly agitated to disperse the spores without detaching mycelial fragments and the suspension shall be gently decanted and filtered through a thin layer of sterile glass wool or through a micro filter funnel with a pore size from $40 \mu\text{m}$ to $100 \mu\text{m}$ into a sterilized centrifuge tube.

The filtered spore suspension shall be centrifuged and the supernatant liquid shall be discarded. The residue shall be re-suspended in not less than 10 ml of sterilized distilled water and centrifuged again. The spores shall be washed in this manner three times.

6.2.2 Preparation for test variant 1

Dilute the final spore residue of each culture in a volume of

- mineral salt solution in accordance with 6.3 but without sucrose (saccharose) if the relevant specification prescribes visual inspection only (see 5.1);
- sterilized distilled water if the relevant specification prescribes checking the function or measuring electrical properties (see 5.1);

that adjusting the spore concentration to 1×10^6 to 2×10^6 /ml determined with a counting chamber or by turbimetry.

Blend equal volumes of the single suspensions sufficient for the relevant inoculation procedure to obtain the final mixed spore suspension ready for inoculation. Spore suspension in mineral salt solution shall be used within 48 h after preparation. Spore suspension in sterilized distilled water shall be used within 6 h after preparation.

NOTE Prepare total volumes of about 100 ml for inoculation by spraying or of about 500 ml for inoculation by painting or dipping.

6.2.3 Preparation for test variant 2

Dilute the final spore residue of each culture in such a volume of nutritive solution in accordance with 6.3 adjusting the spore concentration to 1×10^6 to 2×10^6 /ml determined with a counting chamber or by turbimetry.

Blend equal volumes of the single suspensions sufficient for the relevant inoculation procedure to obtain the final mixed spore suspension ready for inoculation. Use the spore suspension within 6 h after preparation.

NOTE See 6.2.2.

6.3 Control strips

The control strips called for in the test shall consist of strips of pure white filter paper or untreated cotton textile.

The nutritive solution called for in preparing the control strips shall consist of a solution of the following reagents in distilled water.

| Reagent | g/l |
|---|------|
| Potassium dihydrogen phosphate (KH_2PO_4) | 0,7 |
| Dipotassium hydrogen phosphate (K_2HPO_4) | 0,3 |
| Magnesium sulphate ($\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$) | 0,5 |
| Sodium nitrate (NaNO_3) | 2,0 |
| Potassium chloride (KCl) | 0,5 |
| Ferrous sulphate ($\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$) | 0,01 |
| Sucrose (saccharose) | 30,0 |

The pH shall be 6,0 to 6,5 at 20 °C. It shall be adjusted with 0,01 molar NaOH if needed. The solution shall be sterilized in an autoclave at (120 ± 1) °C for 20 min.