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Note d'introduction

Introductory note

This document has been developed by the convener Mr. Klaus Efer, since his appointment at the TC 104 meeting in Frankfurt in April 2002 and by e-mail with the experts in WG 10.

ATTENTION	ATTENTION
CDV soumis en parallèle au vote (CEI) et à l'enquête (CENELEC)	**Parallel IEC CDV/CENELEC Enquiry**

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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 normative documents contain provisions which, through reference in this text, constitute provisions of this part of IEC 60068. At the time of publication, the editions indicated were valid. All normative documents are subject to revision, and parties to agreements based on this part of IEC 60068 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. Members of IEC and ISO maintain register of currently valid International Standards.

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

MIL–STD–810 F: 2000 Method 508.5 Fungus

EN ISO/IEC 17025: 2000 General requirements for the competence of testing and calibration laboratories

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

3 General description

3.1

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.

3.2

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.

3.3

Surface contamination in the form of dusts, splashes, 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.

3.4

Due to the difficulty of maintaining the necessary conditions in a very large chamber, a large composite equipment will normally 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 need to be tested.

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 determining

- the extent of mould growth by visual inspection
- 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 extented 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 determining

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

Note:

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

– Due to the application of nutrients mould growth shall be in exist if not a fungicidal effect shall be considered.

6 Reagents and materials

6.1 Cultures or spores – Supply and conditions

6.1.1

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. But the spores of all cultures shall be used together in a mixed supsension whatever the nature of the specimen.

6.1.2

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

6.1.3

Cultures and freeze–dried spores shall be handled and stored in accordance with the re–commendations 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.

6.1.4 Test fungi

Table 1

No.	Name	Strain No. 3)	Attack to	Note
1	Aspergillus niger	ATCC 6275	many materials	1) 2)
2	Aspergillus terreus	ATCC 10690	plastic materials	1) 2)
3	Chaetomium globosum	ATCC 6205	cellulose	1) 2)
4	Hormoconis resinae	DSM 1203	Hydrocarbon based lubricants	–
5	Paecilomyces varioti	ATCC 18502	plastics and leather	1) 2)
6	Penicillium funiculosum	ATCC 36839	many materials especially textiles	1) 2)
7	Scopulariopsis brevicaulis	ATCC 36840	rubber	1) 2)
8	Trichoderma virens	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

6.1.5

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 ± 1) °C.

Note:

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

6.1.6

If the cultures are not for immediate use, they shall be stored in a refrigerator at a temperature between 5 °C and 10 °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.

6.1.7

The closure of the container shall not be removed until the preparation of the spore suspension will be started. Only one suspension shall be made from the opened container.

6.2 Preparation of spore suspension

6.2.1 General

At first the suspension shall be prepared in sterilized distilled water, to which has been added 0,05 % of a wetting agent. An agent based on N–methyl–taurine or on dioctyl–sodium sulpho-succinate 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 scrape gently 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 to 100 µm 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 such a volume of

- mineral salt solution in accordance with sub–clause 6.3.2 but without sucrose (saccharose) if the relevant specification prescribes visual inspection only (see sub–clause 5.1)
- sterilized distilled water if the relevant specification prescribes to check the function or to measure electrical properties (see sub–clause 5.1)

that adjusting the spore concentration at $1 \cdot 10^6$ to $2 \cdot 10^6$ / 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 e. g.

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 sub–clause 6.3.2 that adjusting the spore concentration at $1 \cdot 10^6$ to $2 \cdot 10^6$ / 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 hours after preparation.

Note: See sub–clause 6.2.2

6.3 Control strips

6.3.1

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

6.3.2

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 ($MgSO_4 \cdot 7\ H_2O$)	0,5
Sodium nitrate ($NaNO_3$)	2,0
Potassium chloride (KCl)	0,5
Ferrous sulphate ($FeSO_4 \cdot 7\ H_2O$)	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 m NaOH if needed.
The solution shall be sterilized in an autoclave at (120 ± 1) °C for 20 minutes.

6.3.3

Immediately before inoculated (see sub–clause 11.2) the control strips shall be saturated by the nutritive solution removed from it and allowed to drain free of drips.

7 Description of test apparatus

7.1 Inoculation by spraying

Preferably a supersonic aerosol apparatus as to be used for therapeutic treatment by inhalation should be used in connection with a safety inoculation chamber (see Annex B).

7.2 Incubation of small specimens

7.2.1

Containers of glass or plastic with lids provided with devices for putting on or suspending the specimens and control strips shall be used.

The container shall be of such size and shape as to expose a sufficient surface area of free water in the base at all times in order to maintain a value of relative humidity greater than 90 % within it.

The devices for putting on or suspending shall be such as to ensure that specimens and control strips are not allowed to touch or to be splashed by the water.

7.2.2

The containers shall be placed in a chamber maintaining an uniform temperature throughout the working space within the range of 28 °C to 30 °C for incubating the specimens and control strips. Any periodic cycling of temperature due to action of the thermostat shall not exceed 1 °C / h.

7.3 Incubation of large specimens

7.3.1

A suitable humidity chamber shall be used for incubation specimens too large for the containers specified in sub–clause 7.2.1. The humidity chamber shall have a well–sealed door to prevent exchange of atmosphere between its interior and the laboratory.

7.3.2

The relative humidity within the working space shall be maintained at a value greater than 90 %. No condensed water from the walls or roof of the chamber shall be allowed to fall on the specimens and control strips. The temperature throughout the working space shall be maintained uniformly within the range of 28 °C to 30 °C. Any periodic cycling of the temperature due to action of the thermostat shall not exceed 1 °C / h.

In order to achieve the specified humidity and temperature uniformly throughout the working space it may be necessary to use forced circulation of the air within it. The flow rate shall not exceed 1 m/s over the surface of the specimen(s).

8 Severities

The severity for each test variant is determined by the duration of the incubation.

Test variant 1 – severity 1 28 days
– severity 2 56 days

as required in the relevant specification.

Test variant 2 – severity 28 days

9 Initial examinations

The specimens shall be visually inspected and shall be electrically and mechanically checked as required by the relevant specification.

10 Pre–conditioning

10.1 Cleaning

The specimens shall be used for the test in the condition as received. Normally they shall not be submitted any special cleaning.

If prescribed by the relevant specification half of the lot of specimens shall be cleaned by washing with Ethanol or demineralized water containing a detergent followed by rinsing with demineralized

water free of detergent and the other half shall remain as received. By this means any mould growth caused by the use of unsuitable materials in construction of the product can be distinguished from that due to surface contamination.

When the grade 0 is required in the relevant specification (Test variant 1), consideration should be given to the need to clean specimens because presence of contamination may promote mould growth.

Note: Grade 0 see sub–clause 12.3

10.2 Damp heat storage

The specimen(s) shall be stored under the conditions of incubation at (29 ± 1) °C and a relative humidity > 90 % and < 100 % for at least 4 h immediately before inoculation.

11 Conditioning

11.1 Application

For the test variant stated in the relevant specification the application shall be carried out according to the method described below.

Test variant 1

If the relevant specification requires checks of functioning and / or measurement of electrical properties two groups of specimens shall be involved:

– Group 1 test specimen(s) inoculated with the spore suspension and incubated

– Group 2 negative control specimen(s) sprayed or painted with or dipped in sterilized distilled water in accordance with the inoculation method used for group 1 and stored at the same temperature and relative humidity as prescribed for incubation but in a sterile environment.

If the relevant specification requires no checks of functioning and / or measurement of electrical properties the group 1 shall be used only.

Test variant 2

Two groups of specimens shall be involved :

– Group 1 test specimens inoculated with spores suspended in the nutritive solution and incubated

– Group 2 in accordance with test variant 1 group 2

Note: Negative control specimen(s)

Negative control specimens should be exposed to the specified conditions in a separate chamber to that in which the inoculated specimens are held. To ensure that no mould grows on the negative control specimens, the chamber should be sterilized by one of the methods given in Clause D 1.1 of Annex D. The test is valid unless both the test specimen(s) and a negative control supports growth.

11.2 Inoculation

The inoculation of the test specimen(s) and control strips (sub–clause 6.3) with the spore suspension (sub–clause 6.2) shall be carried out by spraying if not otherwise prescribed in the relevant specification.