
**Optics and photonics —
Environmental test methods —**

**Part 11:
Mould growth**

*Optique et photonique — Méthodes d'essais d'environnement —
Partie 11: Moisissures*

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 172, *Optics and photonics*, Subcommittee SC 1, *Fundamental standards*.

This second edition cancels and replaces the first edition (ISO 9022-11:1994), of which it constitutes a minor revision.

ISO 9022 consists of the following parts, under the general title *Optics and photonics — Environmental test methods*:

- Part 1: *Definitions, extent of testing*
- Part 2: *Cold, heat and humidity*
- Part 3: *Mechanical stress*
- Part 4: *Salt mist*
- Part 6: *Dust*
- Part 7: *Resistance to drip or rain*
- Part 8: *High internal pressure, low internal pressure, immersion*
- Part 9: *Solar radiation and weathering*
- Part 11: *Mould growth*
- Part 12: *Contamination*
- Part 14: *Dew, hoarfrost, ice*
- Part 17: *Combined contamination, solar radiation*
- Part 20: *Humid atmosphere containing sulfur dioxide or hydrogen sulfide*

- *Part 22: Combined cold, dry heat or temperature change with bump or random vibration*
- *Part 23: Low pressure combined with cold, ambient temperature and dry and damp heat*

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Introduction

Optical instruments are affected during their use by a number of different environmental parameters which they are required to resist without significant reduction in performance and to remain within defined specifications.

The type and severity of these parameters depend on the conditions of use of the instrument (for example, in the laboratory or workshop) and on its geographical location. The environmental effects on optical instrument performance in the tropics and subtropics are totally different from those found when they are used in arctic regions. Individual parameters cause a variety of different and overlapping effects on instrument performance.

The manufacturer attempts to ensure, and the user naturally expects, that instruments will resist the likely rigours of their environment throughout their life. This expectation can be assessed by exposure of the instrument to a range of simulated environmental parameters under controlled laboratory conditions. The severity of these conditions is often increased to obtain meaningful results in a relatively short period of time.

In order to allow assessment and comparison of the response of optical instruments to appropriate environmental conditions, ISO 9022 contains details of a number of laboratory tests which reliably simulate a variety of different environments. The tests are based largely on IEC standards, modified where necessary to take into account features special to optical instruments.

As a result of continuous progress in all fields, optical instruments are no longer only precision-engineered optical products, but, depending on their range of application, also contain additional assemblies from other fields. For this reason, the principal function of the instrument is to be assessed to determine which International Standard should be used for testing. If the optical function is of primary importance, then ISO 9022 is applicable, but if other functions take precedence then the appropriate International Standard in the field concerned should be applied. Cases can arise where application of both ISO 9022 and other appropriate International Standards will be necessary.

Optics and photonics — Environmental test methods —

Part 11: Mould growth

WARNING — Although the species of fungi selected for testing do not normally present a hazard to humans, certain people can develop allergies or other reactions. The use of experienced and trained personnel is required to ensure the proper handling of fungi and the appropriate conduct of the tests. It is, therefore, recommended to entrust the performance of the tests required by this part of ISO 9022 to a microbiological laboratory, since such laboratories have the appropriate equipment and trained personnel.

1 Scope

This part of ISO 9022 specifies the methods relating to the environmental tests of optical instruments, including additional assemblies from other fields (e.g. mechanical, chemical, and electronic devices) under equivalent conditions, for their ability to resist the influence of mould growth.

However, complete instruments or assemblies are only tested as specified in this part of ISO 9022 in exceptional cases. Normally, representative specimens such as mounted optics, material samples, or surface coatings on representative samples are used for testing.

The tests described in this part of ISO 9022 are designed for the selection of materials and components for instruments likely to be used in an environment that is conducive to mould growth, rather than for regular production control.

The purpose of testing is to investigate to what extent the optical, climatic, mechanical, chemical and electrical (including electrostatic) performance characteristics of the specimen are affected by mould growth.

In addition, the tests in this part of ISO 9022 are designed to assess to what extent metabolic waste products (such as enzymes or acids) excreted by fungi, cause etching, corrosion, or short-circuits on, for instance, printed circuit boards.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 9022-1, *Optics and photonics — Environmental test methods — Part 1: Definitions, extent of testing*

3 General information and test conditions

3.1 Test fungi

The species of fungi selected for testing (see [Table 1](#)) are frequently found on optical glass surfaces. Among them are species of hydrophyl, mesophyl, and xerophyl fungi.

Table 1 — Test fungi

Series No.	Species
1	<i>Aspergillus niger</i>
2	<i>Aspergillus flavus</i>
3	<i>Aspergillus versicolor</i>
4	<i>Trichoderma viride</i>
5	<i>Penicillium funiculosum</i>
6	<i>Penicillium citrinum</i>
7	<i>Paecilomyces</i>
8	<i>Chaetomium globosum</i>
9	<i>Eurotium tonophilum</i>
10	<i>Aspergillus penicilloiden (Vitrocolae)</i>

Since strains of fungi change their characteristics with time, only fungal species are specified. The test report or relevant specification, respectively, shall, however, specify the fungal strains used for testing.

3.2 Fungal spore suspension

3.2.1 Fungal cultures

Pure cultures of each of the fungus species specified in [Table 1](#) shall be maintained separately on an appropriate agar medium (e.g. malt agar).

The fungal cultures used for the spore suspension shall not be older than 14 days to 21 days and shall not be used more than once for preparing a mixed spore suspension.

3.2.2 Spore suspensions

For preparing the spore suspensions, and wherever else in this Subclause “water” is specified, use distilled or fully demineralized sterile water containing 0,05 % (mass fraction) of a non-toxic wetting agent such as sodium dioctylsulfosuccinate or sodium laurylsulfate.

Pour 10 ml of the water into each of the fungal cultures described in [3.2.1](#).

Using a sterile platinum loop or any other suitable means, carefully scrape the spores from the mycelial mat. Take care to leave out clumps of agar. Pour the spores charge into a sterile Erlenmeyer flask containing 45 ml of water. Add sterile solid glass beads and shake vigorously to liberate the spores from the fruiting body and to break the spore clumps. Filter the dispersed fungal spore suspension through sterile glass wool to remove mycelial fragments.

Centrifuge the filtrate and discard the supernatant liquid. Resuspend the residue in 50 ml of water and centrifuge. Wash the spores obtained from each of the fungi in this manner three times.

Dilute the final washed residue with the mineral salts solution specified in [Table 2](#) in such a manner that each resultant spore suspension contains $(1\ 000\ 000 \pm 200\ 000)$ spores per millilitre, measured using a suitable counting chamber.

Table 2 — Mineral salts solution

Component	Mass g
Potassium dihydrogen orthophosphate (KH ₂ PO ₄)	0,7
Potassium monohydrogen orthophosphate (K ₂ HPO ₄)	0,7
Magnesium sulfate heptahydrate (MgSO ₄ ·7H ₂ O)	0,7
Ammonium nitrate (NH ₄ NO ₃)	1,0
Sodium chloride (NaCl)	0,005
Iron(II) sulfate heptahydrate (FeSO ₄ ·7H ₂ O)	0,002
Zinc sulfate heptahydrate (ZnSO ₄ ·7H ₂ O)	0,002
Manganese(II) sulfate monohydrate (MnSO ₄ ·H ₂ O)	0,001
Distilled water (H ₂ O)	1 000,0

Sterilize the mineral salts solution in an autoclave at 120 °C for 20 min. Using sodium hydroxide solution, $c(\text{NaOH}) = 0,01 \text{ mol/l}$, adjust the pH of the solution to 6,0 to 6,5 after sterilization. (Percentage purity of the chemicals: atomic adsorption spectroscopy.)

Inoculate each of 10 Petri dishes containing an appropriate agar medium (e.g. malt agar) with spore suspension and immediately incubate the dishes, to check the viability of each fungus species, in the incubation chamber to be used for exposing the specimens. In the event that fungicide-treated specimens are under test in the incubation chamber, expose the Petri dishes to exactly the same climatic conditions in a separate incubation chamber. The absence of growth of any of the various fungus species, at the end of one week, will invalidate the results of all simultaneously performed tests using these spores. Such invalidated tests shall be repeated using freshly prepared mixed spore suspensions from new cultures.

3.2.3 Mixed spore suspension

After having taken the inoculum from the spore suspensions for the purpose described in [3.2.2](#), blend equal portions of the 10 spore suspensions to obtain the final mixed spore suspension.

The spore suspensions from the individual cultures as well as the mixed spore suspension shall be used on the day of their preparation. On no account shall they be stored for later use.

3.3 Control strips

Along with the specimens place at least three control strips in the exposure chamber in order to ensure that optimal climatic conditions are present in the incubation or climatic chamber during exposure of the contaminated specimens. The control strips are of no use if the specimens have been previously treated with fungicides; since these become active predominantly during the volatile phase, a fungicidal atmosphere would develop within the test chamber and hamper the fungal growth on the control strips. In such cases, only the separately incubated individual fungal cultures can be used as control.

The control strips shall be of white sterilized filter paper and shall be of the same size as the specimen (see [3.4](#)). Dip the control strips into the nutrient solution specified in [Table 3](#) and hang them to dry in a sterile atmosphere. Freshly prepare the nutrient solution immediately before impregnating the control strips. Use the control strips on the day of their preparation.

Using hydrochloric acid (HCl) or sodium hydroxide (NaOH) solution, adjust the pH of the solution to 5,3.

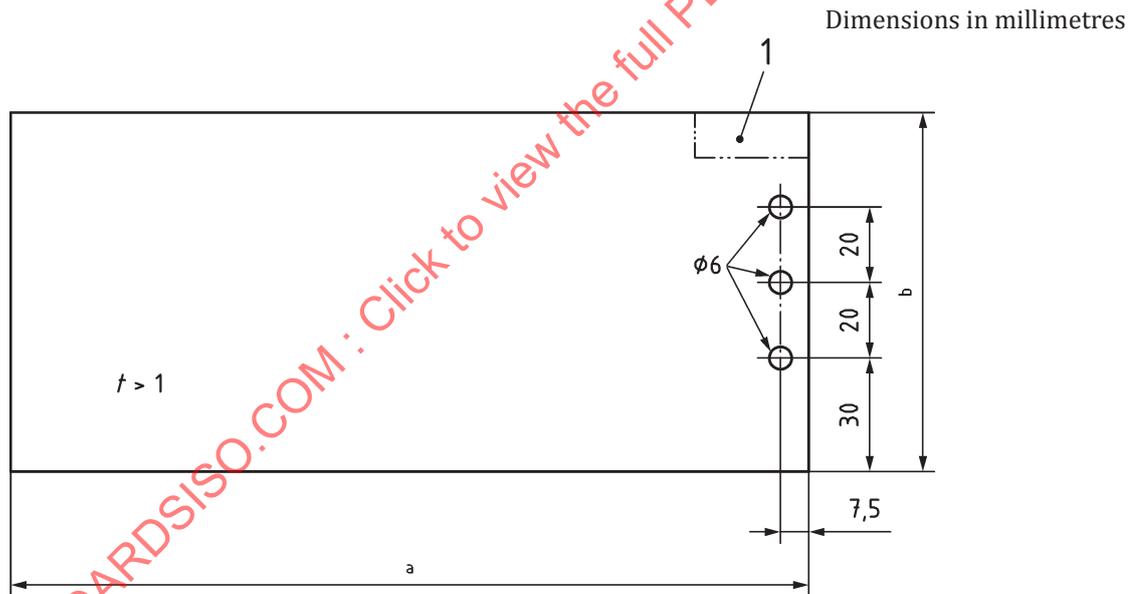
Table 3 — Nutrient solution for soaking control strips

Component	Mass g
Potassium dihydrogen orthophosphate (KH ₂ PO ₄)	0,1
Ammonium nitrate (NH ₄ NO ₃)	0,1
Magnesium sulfate heptahydrate (MgSO ₄ ·7H ₂ O)	0,025
Yeast extract	0,05
Glycerol [C ₃ H ₅ (OH) ₃]	10,0
Distilled water (H ₂ O)	90,0

3.4 Specimen

Unless the testing of complete instruments or assemblies is required in the relevant specification, representative samples shall be used for testing. Sample sheets of the size and dimensions shown in [Figure 1](#) and of at least 1 mm thickness should preferably be used as representative samples of non-metallic coatings or lubricants to be tested.

NOTE Sample sheets of 140 mm ± 2 mm or 280 mm ± 2 mm in length can also be indicated in the appropriate specification.



- Key**
- 1 marking area (on the back)
 - a 210 ± 2 (280 ± 2)
 - b 95 ± 5 (140 ± 2)

Figure 1 — Sample sheet

The coating under test (e.g. varnish) shall be of the same structure as the coating intended for the instrument or for parts of the instrument.

Prior to applying the coating, prepare the surface of the sample sheet in the same manner as required for the original instrument. The coating shall completely surround the sample sheet so as to cover particularly edges, corners, and the edges of holes. The coating shall not be degraded by identification marking; numbers, etc. shall be punched prior to applying the coating.

Sterilize sample sheets at a temperature of 180 °C to 200 °C before applying lubricants as a thin film on one side only.

Use glass hooks or polyamide threads for suspending the specimens in the exposure chamber.

3.5 Incubation cabinets and climatic chambers

The specimens inoculated with the mixed spore suspension shall be incubated in incubation cabinets or climatic chambers under the climatic conditions specified in [Clause 4](#).

The incubation cabinets for climatic chambers shall be adjustable to $29\text{ °C} \pm 1\text{ °C}$ and the temperature may vary in time by $\pm 0,5\text{ K}$ per hour. The cabinets or chambers shall be moisture-proof and shall be provided with a pressure-relief capillary tube or a valve in order to prevent, during exposure, the build-up of pressure within the test chamber.

Normally, there shall be no air circulation during exposure. In the event that the relevant specification does require air circulation, the air flow velocity shall not exceed 0,5 m/s.

Use distilled, demineralized, or deionized water for humidification. A saturated solution (with an ample solid phase) of potassium sulfate (K_2SO_4), covering the entire bottom of the test chamber, can be used to maintain the required relative humidity, which will reach 96 % above the solution.

Moisture shall not be allowed to condense on the specimens during exposure in order to prevent the mixed spore suspension applied to the specimens from being washed off. For the same reason, the specimens shall be protected from condensed-moisture drops falling from the chamber walls.

The contact areas of fixtures supporting the specimen shall be kept to a minimum.

The size of the test chamber and the arrangement of the specimens shall be selected so as to ensure uniform conditioning and aeration of all specimens.

If it is necessary continuously to record the test chamber temperature and relative humidity during exposure, a method shall be specified in the relevant specification.

4 Conditioning method 85: Mould growth

See [Table 4](#).

Table 4 — Degrees of severity for conditioning method 85: Mould growth

Degree of severity	01	02
Exposure time days	28	84
Temperature °C	29 ± 1	
Relative humidity %	96 ± 2	
Number of spores per cm ² of specimen surface	$15\ 000 \pm 3\ 000$	
State of operation	1	

5 Procedure

5.1 General

The test shall be conducted in accordance with the requirements of the relevant specification and with ISO 9022-1.

5.2 Preconditioning

Unless otherwise specified in the relevant specification, use water containing a wetting agent (in accordance with [3.2.2](#)) to clean the specimens, then hang them to dry. Care shall be taken during cleaning not to leave any fragments of the cleaning material (such as cloth or cottonwool) on the specimen. Prior to and during testing, handle the specimens so as not to contaminate them by fingerprints or in any other way. Apply lubricants to the specimens (see [3.4](#)) immediately before exposure.

Then inoculate the specimens and at least three control strips ([3.3](#)) with the mixed suspension (see [3.2.3](#)) by spraying the suspension on the specimens and control strips with an atomizer ensuring a uniform distribution of $(15\ 000 \pm 3\ 000)$ spores per cm^2 of the surface of the specimens.

Place the specimens and the control strips in the incubation cabinet or conditioning chamber not later than 15 min after inoculation. The test chamber shall have been operated at the required climatic conditions at least for 4 h before starting the incubation of the specimens.

In the event that the relevant specification not only requires the evaluation of the mould growth but also evaluation of potential corrosion caused by the mould growth and measurement of the transmission of optical instruments, as many identical non-inoculated specimens as inoculated specimens shall be exposed to identical climatic conditions in separate incubation cabinets or climatic chambers. This is the only known method by which it is possible to distinguish between damage caused by mould growth and damage caused by climatic stress.

5.3 Activities during conditioning

Inspect control strips and Petri dishes for mould growth after seven days of conditioning in accordance with [Clause 4](#). If there is little or no growth on the control strips and/or the Petri dishes, the entire test is unacceptable and shall be repeated.

During conditioning, open the incubation cabinet or conditioning chamber once a week for a few seconds, for the purpose of air renewal. At the end of the exposure time, the mould growth on the control strips shall be more substantial in density than on the seventh day of conditioning; otherwise, the test is unacceptable and shall be repeated.

5.4 Recovery

Unless otherwise specified in the relevant specification, evaluate the specimens at the end of the conditioning period without leaving them to dry first. On no account shall specimens be cleaned prior to evaluating the mould growth. If a comparative corrosion evaluation is required, carefully remove the mycelia, using water and soft cloth, after the evaluation of the mould growth.

5.5 Evaluation

For the evaluation of the extent of mould growth, refer to [Table 5](#). If, in addition to mould growth evaluation, evaluation of corrosion damage due to mould growth is required, remove the mycelia and compare the specimens with the non-inoculated specimens.

5.6 General level for acceptance

Unless otherwise specified in the relevant specification, the test shall be accepted as successful if the amount of growth is less than or equal to grade 2 in [Table 5](#).