
**Building and civil engineering
sealants — Assessment of the fungal
growth on sealant surfaces**

*Mastics pour le bâtiment et le génie civil — Évaluation de la
croissance fongique à la surface des mastics*

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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).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 59, *Buildings and civil engineering works*, Subcommittee SC 8, *Sealants*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Sealants used in high humidity environments experience growth of microorganisms; and it can be necessary to test the function of sealant under normal service conditions to ensure that the sealant surface remains free of the growth microorganisms over a designated function period. This test is designed to evaluate the 5 most common strains of microorganisms found on sealant surfaces.

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Building and civil engineering sealants — Assessment of the fungal growth on sealant surfaces

1 Scope

This document specifies a method for the evaluation of the fungal growth on sealants which are used in joints in building construction.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 6927, *Building and civil engineering sealants — Vocabulary*

ISO 846, *Plastics — Evaluation of the action of microorganisms*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6927 and ISO 846 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

4 Apparatus

4.1 Incubators, used for tests involving fungal attack, shall be capable of controlling the temperature to ± 1 °C at any temperature from 20 °C to 35 °C. A relative humidity of 90 % or greater one needs to be ensured during incubation.

4.2 Oven, capable of controlling the temperature at (45 ± 2) °C for drying test specimens.

4.3 Water bath or ventilated oven, capable of being controlled at (50 ± 1) °C for heating the specimen during the water immersion.

4.4 Autoclave, capable of maintaining a temperature and pressure of (120 ± 2) °C and 2 bar, respectively, for sterilizing Petri dishes.

4.5 Analytical balance, accurate to 0,1 mg.

4.6 Laboratory centrifuge.

4.7 Stereoscopic microscope, magnification $\times 50$.

4.8 Glass or plastic disposable Petri dishes, of suitable size for exposing test specimens.

4.9 Distilled or deionized water, used for the preparation of all solutions and nutritive media and for all determinations, which shall be distilled or deionized and have a conductivity of $< 1\mu\text{S}/\text{cm}$.

4.10 Ethanol-water mixture, in the proportions, by mass, of 70:30.

4.11 Glass containers, with a volume of about 2 000 ml. The containers shall have a cover to avoid evaporation.

4.12 Anti-adherent, PE or Polytetrafluoroethylene (PTFE) with thickness of $(2 \pm 0,5)$ mm and a width of minimum (50 ± 4) mm.

5 Test fungi preparation

5.1 Test fungi

The test fungi shall be obtained from national culture collections. The strains to be used are listed in [Table 1](#).

Table 1 — Test fungi strains

Name	Strain
<i>Fusarium oxysporum</i>	ATCC 7808 or other corresponding national strain (e.g. CBS 267,50, DSM 841)
<i>Aspergillus niger</i>	ATCC 6275 or other corresponding national strain
<i>Phoma herbarum</i>	ATCC 12569 or other corresponding national strain ISO 21265:2021 (e.g. IMI 49948)
<i>Exophiala jeanselmei</i>	ATCC 34123 or other corresponding national strain (e.g. CBS 507,90, CBS 664,76, Duke 2405, IFM 4852, IHM 283, NCPF 2439)
<i>Penicillium chrysogenum</i>	ATCC 10106 or other corresponding national strain (e.g. CBS 306,48, IMI 24314)

If there are technical reasons, and by agreement between the interested parties, other species may be used. In this case, the strains used shall be stated in the test report.

5.2 Stock strains

Culture the test fungi ([5.1](#)) in tubes on agar slants of the following composition:

- Oatmeal 20 g
- Malt extract 10 g
- Agar 20 g
- Water 1 000 ml

Adjust the pH to $5,5 \pm 0,2$ with 0,01 mol/l HCl solution. Sterilize the agar composition at (120 ± 2) °C for 20 min in an autoclave in an atmosphere saturated with water vapour.

After incubation at (29 ± 1) °C, well sporulating cultures may then be used. They shall not be stored more than 4 weeks at this temperature.

Because of the possibility of genetic and physiological changes in the test fungi during culturing on artificial media, the intervals between subculturing shall be reduced to a minimum by suitable measures (e.g. lyophilisation of cultures, storage at +4 °C or in liquid nitrogen).

5.3 Solutions and nutritive media

5.3.1 Stock mineral-salt solution is of the following composition (use only chemicals of analytical grade or equivalent purity):

- NaNO_3 2,0 g
- KH_2PO_4 0,7 g
- K_2HPO_4 0,3 g
- KCl 0,5 g
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0,5 g
- $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0,01 g
- H_2O 1 000 ml

Adjust the pH to 6,0 to 6,5 with sterile 0,01 mol/l NaOH solution.

5.3.2 Mineral-salt/wetting-agent solution is prepared by adding to 1 l of stock mineral-salt solution (5.3.1) 0,1 g of a non-toxic wetting agent such as N-methyltaurine or polyglycol ether and sterilizing in an autoclave at (120 ± 2) °C for 20 min.

5.3.3 Mineral-salt/glucose solution is prepared by adding to stock mineral-salt solution (5.3.1) sufficient glucose to give a concentration of 30 g/l and sterilizing in an autoclave at (120 ± 2) °C for 20 min.

5.3.4 Complete agar medium is prepared by adding to mineral-salt/glucose solution (5.3.3) sufficient agar to give a concentration of 20 g/l. Adjust the pH to between 6,0 and 6,5 at 20 °C with NaOH solution. Sterilize in an autoclave at (120 ± 2) °C for 20 min.

6 Preparation of test specimens

6.1 General

The sealant and the used materials (substrates and anti-adherent) shall be brought to (23 ± 2) °C over 24 h minimum.

6.2 Preparation and conditioning

10 or 20 test specimens shall be prepared. Extrude the sealant on a suitable anti-adherent substrate (e.g. PE or Polytetrafluoroethylene (PTFE)) and prepare a sheet with a thickness of $(2 \pm 0,5)$ mm and a width of minimum (50 ± 4) mm and a length (500 ± 10) mm for 10 test specimens.

The following precautions shall be taken:

- a) the formation of air bubbles shall be avoided;
- b) the sealant shall be pressed on surface of the anti-adherent substrate;
- c) the sealant surface shall be tooled so that a flat surface is obtained.

After preparation, the strap shall be preconditioned for 28 days at (23 ± 2) °C and (50 ± 10) % relative humidity.

Cut 10 or 20 test specimens ([Table 2](#)) from the cured strap while ensuring the exposed surface (top surface) is the testing surface. The test specimens shall have the dimensions (50 ± 4) mm \times (50 ± 4) mm \times $(2 \pm 0,5)$ mm.

Table 2 — Test specimens for four groups

Items	Immersion	Without immersion
Solidified agar medium	5 specimens	5 specimens
Without solid medium (Optional)	5 specimens	5 specimens

6.3 Cleaning, labelling and storage of specimens

6.3.1 Cleaning

Dip the specimens into an ethanol-water mixture ([4.10](#)) for 1 min. Let the specimen dry. Carry out all subsequent handling of the specimens using forceps to avoid contamination by extraneous matter.

6.3.2 Labelling and storage

Store the cleaned specimens at ambient temperature.

It is advisable to store the specimen in suitable containers (e.g. Petri dishes) and label the Petri dishes, not the specimens, to avoid surface reactions.

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7 Test procedures

7.1 General

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[Figure 1](#) shows the general procedure.