



IPC-TM-650 TEST METHODS MANUAL

1 Scope The fungus resistance test is used to determine the resistance of materials to fungi and to determine if such material is adversely affected by fungi under conditions favorable for their development, namely high humidity, warm atmosphere, and presence of inorganic salts.

2 Applicable Documents None

3 Test Specimen Specimens must be a minimum size of 50 mm x 50 mm [1.97 in x 1.97 in] with copper foil (if applicable) removed by etching using standard commercial practices.

4 Apparatus and Reagents

4.1 Test Chamber The incubator shall be capable of maintaining 30 ± 1 °C [86 ± 2 °F] and 95 ± 2% relative humidity and have an ultraviolet (360 nm) source for subsequent decontamination. Provisions shall be made to prevent condensation from dripping on the test item. There shall be free circulation of air around the test item and the contact area of fixtures supporting the test item shall be kept to a minimum.

4.2 Sterilizer

4.3 Centrifuge

4.4 pH Meter

4.5 Colony Counter

4.6 Incubator

4.7 Dishwasher

4.8 Petri Dishes

4.9 Filter Paper

4.10 Media Solutions

4.11 Microorganisms

4.12 Atomizer, 15,000 ± 3000 spores

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Originating Task Group Solder Mask Performance Task Group (5-33b)	

5 Procedures

5.1 Preparation of Test Media

5.1.1 Mineral-Salts Solution

Prepare the solution to contain the following:

Potassium dihydrogen orthophosphate (KH ₂ PO ₄)	0.7g
Potassium monohydrogen orthophosphate (K ₂ HPO ₄)	...	0.7g
Magnesium sulfate heptahydrate (MgSO ₄ •7H ₂ O)	0.7g
Ammonium Nitrate (NH ₄ NO ₃)	1.0g
Sodium chloride (NaCl)	0.005g
Ferrous sulfate heptahydrate (FeSO ₄ •7H ₂ O)	0.002g
Zinc sulfate heptahydrate (ZnSO ₄ •7H ₂ O)	0.002g
Manganous sulfate monohydrate (MnSO ₄ •H ₂ O)	0.001g
Distilled water	1000 ml

Sterilize the mineral salt solution by incubating at 121 °C [250 °F] for a minimum of 20 minutes. Adjust the pH of the solution by the addition of 0.01 normal solution of NaOH so that after sterilization the pH is between 6.0 and 6.5. Prepare sufficient salt solutions for the required tests.

5.1.2 Purity of Reagents Reagent grade chemicals shall be used in all tests. Unless otherwise specified, it is intended that all reagents shall conform to the specification of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

5.1.3 Purity of Water Unless otherwise specified, references to water shall be understood to mean distilled water or water of equal purity.

5.1.4 Preparation of Mixed Spore Suspension

The following test fungi shall be used:

Description	ATCC
Aspergillus niger	9642
Chaetomium globosum	6205
Gliocladium virens	9645
Aureobasidium pullulans	9348
Penicillium funiculosum	9644

5.1.5 Maintain cultures of these fungi separately on an appropriate medium such as potato dextrose agar. However, the culture of Chaetomium globosum shall be cultured on

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strips of filter paper on the surface of mineral salts agar. (Mineral salt agar is identical to mineral salt solution, but contains an additional 15.0 g of agar per liter.)

5.1.6 The stock cultures may not be kept longer than four months at 6 ± 4 °C [43 ± 7 °F] at which time subcultures shall be made and new stocks shall be selected from the subcultures.

5.1.7 If genetic or physiological changes occur in the culture, obtain new cultures in accordance with 5.1.4. Subcultures used for preparing new stock cultures or the spore suspension shall be incubated at 30 °C \pm 1 °C [86 °F \pm 2 °F] for at least nine days.

5.1.8 Preparation of Spore Suspension

5.1.8.1 Pour 10 ml of a sterile solution containing about 0.05 g/L of a nontoxic wetting agent such as sodium dioctyl sulfosuccinate or sodium lauryl sulphate into one culture of each fungus.

5.1.8.2 Use a sterile platinum or nichrome inoculating wire to free the fungus from the culture medium by gently scraping the surface growth of the agar from the culture of the medium.

5.1.8.3 Pour the spore suspension into a sterile 125-ml glass-stoppered Erlenmeyer flask containing 45 ml of sterile water and 50 to 75 solid glass beads, 5.0 mm [0.197 in] in diameter.

5.1.8.4 Shake the flask vigorously to liberate the spores from the fruiting bodies and to break the spore clumps.

5.1.8.5 Filter the dispersed fungal spore suspension through a 6 mm layer of glass wool contained in a glass funnel and collect into a sterile flask. This process should remove large mycelial fragments and clumps of agar which could interfere with the spraying process.

5.1.8.6 Centrifuge the filtered spore suspension and discard the supernatant liquid.

5.1.8.7 Resuspend the residue in 50 ml of sterile water and centrifuge.

5.1.8.8 Wash the spores obtained from each of the fungi in this manner three times.

5.1.8.9 Dilute the final washed suspension with sterile mineral-salt solution such that the resultant spore suspension contains $1,000,000 \pm 200,000$ spores per ml as determined with a colony counter.

5.1.9 Repeat the steps in 5.1.8 for each organism used in the test and blend equal volumes of the resultant spore suspension to obtain the final mixed spore suspension. The spore suspension may be prepared fresh each day or may be held at 6 ± 4 °C [43 ± 7 °F] for not more than seven days.

5.2 Viability of Inoculum Control With each daily group of tests, place one piece of sterilized filter paper, 2.5 cm [1.0 in] square, on hardened mineral-salt agar in three separate Petri dishes. Inoculate these dishes with the spore suspension by spraying the suspension from a sterilized atomizer until initiation of droplet coalescence. Incubate these at 30 ± 1 °C [86 ± 2 °F] at a relative humidity not less than 85% and examine them after seven days of incubation. There shall be copious growth on all three of the filter paper control specimens. Absence of such growth requires repetition of the test.

5.3 Control Items

5.3.1 In addition to the viability of inoculum control, known susceptible substrates shall be inoculated along with the test item to insure that proper conditions are present in the incubation chamber to promote fungus growth.

5.3.2 The control items shall consist of 284.5 g/m² [8.25-oz] bleached, scoured, 5 cm [2 in] long cotton duck strips, that have been dipped into a solution containing 10% glycerol, 0.1% potassium dihydrogen orthophosphate (KH₂PO₄), 0.1% ammonium nitrate (NH₄NO₃), 0.025% magnesium sulfate (MgSO₄ 7H₂O), and 0.05% yeast extract (pH 5.3), from which the excess liquid has been removed.

5.3.3 The strips should be hung to air dry before being inoculated and placed into the chamber.

5.4 Inoculation of Test and Control Item

5.4.1 Mount the test and control items on suitable fixtures or suspend from hangers. No cleaning of the test item shall be permitted for 72 hours prior to the beginning of the fungus test. Equipment handling prior to and during the fungus test shall be accomplished without contamination of the equipment.

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5.4.2 Precondition the chamber and its contents at: 30 ± 1 °C [86 ± 2 °F] and $95 \pm 2\%$ relative humidity for at least four hours.

5.4.3 Inoculate the test and control items with the mixed fungus spore suspension (5.1.4) by spraying it on and into the test and control items (if not hermetically sealed) in the form of a fine mist from a previously sterilized atomizer or nebulizer. In spraying the test and control items, care should be taken to spray all surfaces that are exposed during use or maintenance. If the surfaces are nonwetting, spray until initiation of droplet coalescence. Incubation is to be started immediately following the inoculation.

5.5 Test Incubation of Test Items

5.5.1 Incubate test items at an air temperature of 30 ± 1 °C [86 ± 2 °F] at 85% minimum relative humidity.

5.5.2 After seven days, inspect the growth on the control items to be assured that the environmental conditions are suitable for growth. If inspection reveals that the environmental conditions are unsuitable for growth, the entire test shall be repeated.

5.5.3 If the control items show satisfactory fungus growth, continue the test for a period of 28 days from the time of inoculation, or as specified.

5.6 Evaluation

5.6. Report those specimens which were found to be nutrient to fungus growth.

5.6.2 Corrosion should be noted separately from the fungus test results.

6 Notes

6.1 Source for Microorganisms

6.1.1 ATC
P.O. Box 1549
Manassas, VA 20108
(703) 365-2700
www.atcc.org

6.2 Secondary Sources for Microorganisms

6.2.1 Pioneering Research Division
U.S. Army Natick Laboratories
Natick, Massachusetts 01760

6.2.2 USDA, 1815 North University St., Peoria, IL 61604

6.3 After evaluation, the materials and the test chamber must be decontaminated by exposure on all sides to ultraviolet rays (360 nm) for a minimum of two hours, or sprayed with a solution of 1:750 zephiran chloride solution. (One part zephiran chloride to 750 parts distilled water.)

6.4 Safety Observe all appropriate precautions on MSDS for chemicals involved in this test method.

6.5 Nutrient Agar Covered petri dishes containing nutrient agar are considered to have the desired humidity. Covers on larger dishes containing nutrient agar may need to be sealed with masking tape.