ASSOCIATION CONNECTING
ELECTRONICS INDUSTRIES®

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Number	
2.6.1.1	
Subject	
Fungus Resistance – Conformal Coating	
Date	Revision
07/00	
Originating Task Group	
Conformal Coating Task Group, 5-33a	

IPC-TM-650
TEST METHODS MANUAL

1.0 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.

2.0 Applicable Documents None

3.0 Test Specimen Standard laboratory glass slides of 50 mm x 50 mm [2.0 in x 2.0 in] minimum size.

4.0 Apparatus and Reagents

4.1 Test Chamber The autoclave shall be capable of maintaining 30 ± 1°C [86 ± 1.8°F] and 95 ± 2% relative humidity and an ultra violet (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 Micro-organisms

4.12 Atomizer, 15,000 ± 3000 spores

5.0 Procedures

5.1 Preparation of Test Media

5.1.1 Mineral-Salts Solution

Prepare the solution to contain the following:

Potassium dihydrogen orthophosphate(KH_2PO_4) 0.7g
Potassium monohydrogen orthophosphate (K_2HPO_4) ... 0.7g
Magnesium sulfate heptahydrate (Mg $SO_4 \cdot 7H_2O$) 0.7g
Ammonium Nitrate ((NH_4NO_3) 1.0g
Sodium chloride (NaCl) 0.005g
Ferrous sulfate heptahydrate ($FeSo_4 \cdot 7H_2O$) 0.002g
Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) 0.002g
Manganous sulfate monohydrate ($MnSO_4 \cdot H_2O$) 0.001g
Distilled water ..... 1000 ml

Sterilize the mineral salts solution by autoclaving at 121°C [249.8°F] for 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 salts solution 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 virans 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 strips of filter paper on the surface of mineral salts agar. (Mineral salts agar is identical to mineral salts solution, but contains in addition 15.0 g of agar per liter.)

5.1.6 The stock cultures may be kept for not more than four months at 6 ± 4°C [42.8 ± 7.2°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, obtain new cultures as specified above. Subcultures used for preparing new stock cultures or the spore suspension shall be incubated at 30 ± 1°C [86 ± 1.8°F] for nine to 12 days or longer.

5.1.8 Prepare a spore suspension of each of the five fungi by pouring into one subculture of each fungus, a 10-ml portion of a sterile solution containing 0.05 g per liter of a nontoxic wetting agent such as sodium dioctyl sulfosuccinate or sodium lauryl sulfate.

5.1.9 Use a sterile platinum or nichrome inoculating wire to scrape gently the surface growth from the culture of the test organism.

5.1.10 Pour the spore charge into a sterile 125-ml glass-stoppered Erlenmeyer flask containing 45 ml of sterile water and 50 to 75 solid glass beads, 5 mm in diameter.

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

5.1.12 Filter the dispersed fungal spore suspension, through a 6 mm layer of glass wool contained in a glass funnel, into a sterile flask.

5.1.13 This process should remove large mycelial fragments and clumps of agar which could interfere with the spraying process.

5.1.14 Centrifuge the filtered spore suspension aseptically and discard the supernatant liquid.

5.1.15 Resuspend the residue in 50 ml of sterile water and centrifuge. Wash the spores obtained from each of the fungi in this manner three times.

5.1.16 Dilute the final washed residue with sterile mineral-salts solution in such a manner that the resultant spore suspension shall contain 1,000,000 ± 200,000 spores per ml as determined with a counting chamber.

5.1.17 Repeat this operation 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 [42.8 ± 7.2°F] for not more than seven days.

5.2 Viability of Inoculum Control With each daily group of tests, place each of three pieces of sterilized filter paper, 1 inch square, on hardened mineral-salts agar in separate Petri dishes. Inoculate these with the spore suspension by spraying the suspension from a sterilized atomizer until initiation of droplet coalescence. Incubate these at 30 ± 1°C [86 ± 1.8°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 cotton duck 8.25-ounce strips that are 5 cm, that have been dipped intoa solution containing 10% glycerol, 0.1% potassium dihydrogen orthophosphate (KH_2PO_4), 0.1% ammonium nitrate (NH_4NO_3), 0.025% magnesium heptahydrate sulfate ($MgSO_4 \cdot 7H_2O$), and 0.05% yeast extract (pH 5.3), and 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.

5.4.2 Precondition the chamber and its contents at: 30 ± 1°C [86 ± 1.8°F] and 95 ± 2% relative humidity for at least four hours.

5.4.3 Inoculate the test and control items with the mixed fungus spore suspension 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 which 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 under static temperature of 28-30°C [82.4-86°F] with a minimum 85% 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

5.6.1 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.

6.0 *Notes*

6.1 Source for Micro-organisms

6.1.1 American Type Culture Collection
10801 University Blvd.
Manassas, VA 20110-2209 (USA)
703-365-2700
http://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 North University St.
Peoria, IL 61604
Contact: Dr. Stephen Peterson
309-685-4011

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.