METHOD 508.8

FUNGUS

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METHOD 508.8

FUNGUS

NOTE: Tailoring is essential. Select methods, procedures and parameter levels based on the tailoring process described in Part One, paragraph 4.2.2, and Annex C. Apply the general guidelines for laboratory test methods described in Part One, paragraph 5 of this Standard.

1. SCOPE.

1.1 Purpose.

The purpose of this fungus test is to assess the susceptibility of materials to fungal growth. The primary objectives of the fungus test are to determine:

a. If the materials or the assembled combination of same, will support fungal growth, and where possible, identify dominant species present.

b. How rapidly fungus will grow on the materiel.

c. How fungus may affect the materiel, its mission, and its safety for use following the growth of fungus on the materiel.

d. If the materiel can be stored effectively in a field environment.

e. If there are simple cleaning processes.

1.2 Application.

Since microbial deterioration is a function of temperature and humidity, and is an inseparable condition of the a hot, humid environment and the mid-latitudes tropics, consider it in the design of all standard, general-purpose materiel (from paragraph 6.1, reference a). This method is used to determine if fungal growth will occur and, if so, how it may degrade/impact the use of the materiel.

NOTES: 1. This test procedure and the accompanying preparation and post-test analysis involve highly-specialized techniques and potentially-hazardous organisms. Use only technically-qualified personnel (e.g., microbiologists, trained technicians) to perform the test.

2. Although the basic (documented) resistance of materials to fungal growth (Annex B) is helpful in the design of new materiel, it has shown to be unreliable in determining the fungal susceptibility of complex materials. The use of testing by analysis is discouraged. The physical structure of combined materials and the possible contamination of resistant materials during manufacture are beyond the purview of analysis, and necessitate laboratory or natural environment tests to verify the resistance of the assembled materials to fungal growth.

1.3 Limitations.

This test is designed to obtain data on the susceptibility of materiel. This method is not intended for but may be used to test basic materials since various other test procedures, including pure culture, mixed culture, and plate testing are available.

2. TAILORING GUIDANCE.

2.1 Selecting the Fungus Method.

After examining requirements documents and applying the tailoring process in Part One of this Standard to determine where fungal growth is anticipated in the life cycle of materiel, use the following to confirm the need for this Method and to place it in sequence with other methods.

2.1.1 Effects of Fungus Growth.

Fungal growth impairs the functioning or use of materiel by changing its physical properties.

2.1.1.1 Detrimental Effects.

The detrimental effects of fungal growth are summarized as follows:

a. Direct Attack on Materials. Nonresistant materials are susceptible to direct attack as the fungus breaks the materials down and uses them as nutrients. This results in deterioration affecting the physical properties of the material. Examples of nonresistant materials are:

(1) Natural Materials. Products of natural origin are most susceptible to this attack.

(a) Cellulosic materials (e.g., wood, paper, natural fiber textiles, and cordage).

(b) Animal- and vegetable-based adhesives.

(c) Grease, oils, and many hydrocarbons.

(d) Leather.

(2) Synthetic Materials.

(a) PVC formulations (e.g., those plasticized with fatty acid esters).

(b) Certain polyurethanes (e.g., polyesters and some polyethers).

(c) Plastics that contain organic fillers of laminating materials.

(d) Paints and varnishes that contain susceptible constituents.

b. Indirect Attack on Materials. Damage to fungus-resistant materials results from indirect attack when:

(1) Fungal growth on surface deposits of dust, grease, perspiration, and other contaminants (that find their way onto materiel during manufacture or accumulate during service) causes damage to the underlying material, even though that material may be resistant to direct attack.

(2) Metabolic waste products (i.e., organic acids) excreted by fungus cause corrosion of metals, etching of glass, or staining or degrading of plastics and other materials.

(3) The products of fungus on adjacent materials that are susceptible to direct attack come in contact with the resistant materials.

2.1.1.2 Physical Interference.

Physical interference can occur as follows:

a. Electrical or Electronic Systems. Damage to electrical or electronic systems may result from either direct or indirect attack. Fungi can form undesirable electrical conducting paths across insulating materials, for example, or may adversely affect the electrical characteristics of critically adjusted electronic circuits.

b. Optical Systems. Damage to optical systems results primarily from indirect attack. The fungus can adversely affect light transmission through the optical system, block delicate moving parts, etched glass, and change non-wetting surfaces to wetting surfaces with resulting loss in performance.

2.1.1.3 Health and Aesthetic Factors.

Fungus on materiel can cause physiological problems (e.g., allergies) or be so aesthetically unpleasant that the users will be reluctant to use the materiel.

2.1.2 Sequence Among Other Test Methods.

a. General. Use the anticipated life cycle sequence of events as a general sequence guide (see Part One, paragraph 5.5).

b. Unique to This Method. Because of the potentially unrepresentative combination of environmental effects, it is generally inappropriate to conduct this test on the same test sample previously subjected to salt fog, sand and dust, or humidity tests. However, if it is necessary, perform the fungus test before salt fog, or sand and

dust tests. A heavy concentration of salt may affect the germinating fungus growth and sand and dust can provide nutrients, thus leading to a false indication of the bio-susceptibility of the test item. Be sure to decontaminate the test item prior to other testing (see Annex A).

2.2 Selecting Procedure Variations.

This Method has one procedure. Since the combination of temperature and humidity is critical to microbial growth, it is essential that these be maintained as specified in the procedure. However, other possible variations are described below.

2.2.1 Test Duration.

Twenty-eight days is the minimum test period to allow for fungus germination, breakdown of carbon-containing molecules, and degradation of material. Since indirect effects and physical interference are not likely to occur in the relatively short time frame of the fungus test, consider extension of the exposure period to 84 days if a greater degree of certainty (less risk) is required in determining the existence or effect of fungus growth.

2.2.2 Choice of Fungus.

The fungi used for this test are listed in Table 508.8-I. These organisms were selected because of their ability to degrade materials, their worldwide distribution, their stability, and their Biological Safety Level 1 classification. These organisms have, where possible, been identified with respect to the materials to which they are known to attack. Substitution of the species is not recommended.

a. Because the test item may not be sterile before testing, other microorganisms may be present on the surfaces. When the test item is inoculated with the test fungi, both these and the other organisms will compete for available nutrients. It is not surprising to see organisms other than the test fungi growing on the test item at the end of the test. Hence, the need for trained personnel, e.g., mycologists, microbiologists, etc., to identify such situations.

b. The spores chosen were identified as being prolific throughout the world and should remain as the base species for this test. The more dominant species will prevail in testing, therefore adding more species will likely only increase test costs without an increase in deterioration to material. You may add, but not substitute, additional species of fungus to those required in this test Method. However, if additional fungi are used (suggest only 1 or 2 other species), base their selection on prior knowledge of specific material deterioration. Consult trained personnel, e.g., mycologists, microbiologists, etc., to identify such situations.

3. INFORMATION REQUIRED.

3.1 Pretest.

The following information is required to conduct fungus tests adequately.

a. General. Information listed in Part One, paragraphs 5.7 and 5.9; and Annex A, Task 405 of this Standard.

b. Specific to This Method.

(1) Test item composition if known.

(2) Species to be used.

(3) Additional species to be added based upon known materiel composition.

(4) Duration of test.

(5) Test item photographs.

c. Tailoring. Necessary variations in the basic test procedures to accommodate LCEP requirements.

Table 508.8-I. TEST FUNGUS.

FUNGUS	FUNGUS SOURCES IDENTIFICATION NO.[1]			MATERIALS AFFECTED
	NRRL[2]	USDA[3]	ATCC[4]	
Aspergillus flavus *(Aflotoxin not produced)*	NRRL A5244	QM 380	ATCC 9643	Leathers, textiles, rubber. Electrical insulation, varnish, wax, packing materials, etc.
Trichoderma virens	NRRL 2314	QM 365	ATCC 9645	Degrades adhesives, cork, automotive components, electrical insulation, packing materials, plastics, polymers.
Talaromyces pinophilus (pinofilum) *(formerly known as Penicillium funiculosum)*	NRRL 3647	QM 474	ATCC 11797	Textiles, plastics, cotton fabric, polymers, automotive components such as gaskets, distributors, cables, hoses, PVC, airborne equipment such as breakers, solenoids, switches, remote transmission accessories
Chaetomium globosum	NRRL 1870	QM 459	ATCC 6205	Cellulose and any components containing paper and paper products such as packing materials, textiles, polymeric hydrocarbons and some synthetic polymeric materials
Aspergillus brasiliensis (formerly known as niger)	NRRL 3536	QM 386	ATCC 9642	Textiles, vinyl, conformal coatings, etches glass, insulation, leather, etc.; resistant to tanning salts

Notes: 1. A catalogue number used by suppliers to identify various species within their collection.

2. US Department of Agriculture, Northern Regional Research Center, ARS Culture Collection, 1815 North University Street, Peoria, IL 61604.

3. US Department of Agriculture, Northern Regional Research Center, Quartermaster Collection, 1815 North University Street, Peoria, IL 61604.

4. American Type Culture Collection, 10801 University Blvd, Manassas, VA 20110-2209. (All suppliers may distribute the fungus in a lyophilized state or on agar slants. See USDA or ATCC site for any permit information needed for each State).

3.2 During Test.

Collect the following information during conduct of the test:

a. General. Information listed in Part One, paragraph 5.10; and in Annex A, Tasks 405 and 406 of this Standard.

b. Specific to This Method.

(1) Record of chamber temperature and humidity versus time conditions.

(2) Evidence of fungus growth on the cotton control strips at the 7-day check.

(3) Location of any fungal growth.

3.3 Post-Test.

The following post test data shall be included in the test report.

a. General. Information listed in Part One, paragraphs 5.10 and 5.13; and in Annex A, Task 406 of this Standard.

b. Specific to This Method.

(1) Evidence of fungus growth at the end of the test. If growth is found, identify the species if possible.

(2) Narrative description of growth, including colors, areas covered, growth patterns, density of growth, and photographs. (See Table 508.8-II.)

(3) Effect of fungus on performance or use:

(a) As received from the chamber.

(b) After removal of fungus, if appropriate.

(c) Physiological or aesthetic considerations.

(4) Observations to aid in failure analysis.

(5) Any deviation from the original test plan or test request.

4. TEST PROCESS.

4.1 Test Facility.

In addition to the standard requirements for test chambers, the following apply to chambers to be used for fungus tests.

4.1.1 Test Chamber.

Construct the chamber and accessories in a manner to prevent condensation from dripping on the test item. If required, filter-vent the chamber to the atmosphere to prevent the buildup of pressure and release of spores into the atmosphere.

4.1.2 Sensors.

Determine the relative humidity by employing either solid state sensors whose calibration is not affected by water condensation, or by an equivalent method such as fast-reacting wet-bulb/dry-bulb sensors or dew point indicators. Sensors that are sensitive to condensation, such as the lithium chloride type, are not recommended for tests with high relative humidity levels. A data collection system, including an appropriate recording device(s), separate from the chamber controllers is necessary to measure test volume conditions. If charts are used, use charts readable to within ± 2 °C (± 3.6 °F). If the wet-wick control method is approved for use, clean the wet bulb and tank and install a new wick before each test and at least every 30 days. Ensure the wick is as thin as realistically possible to facilitate evaporation (approximately 1/16 of an inch thick) consistent with maintaining a wet surface around the sensor. Use water in wet-wick systems that are of the same quality as that used to produce the humidity. When physically possible, visually examine the water bottle, wick, sensor, and other components making up relative humidity measuring systems at least once every 24 hours during the test to ensure they are functioning as desired.

4.1.3 Air Velocity.

Minimal airflow is recommended for this method. It has been found that velocities greater than 0.5 meters/second (98 feet/minute) retard the growth of fungus. If a wet bulb sensor is used, recommend an air velocity flowing across the wet bulb sensor of not less than 4.6 meters/second (900 feet/minute, or as otherwise specified in sensor response data), and ensure the wet wick is on the suction side of the fan to eliminate the effect of fan heat. Maintain the flow of air anywhere within the envelope of air surrounding the test item between 0.5 and 1.7 meters/second (98 to 335 feet/minute).

4.1.4 Decontamination.

Prior to testing, ensure the chamber is decontaminated in accordance with the guidance at Annex A.

4.2 Controls.

In addition to the information provided in Part One, paragraph 5, the following controls apply to this test.

4.2.1 Relative Humidity.

In addition to the requirements appropriate for Method 507.6 Humidity, and water purity as described in Part One, paragraph 5.16, determine the relative humidity by employing either solid state sensors whose calibration is not affected by water condensation or by an approved equivalent method such as fast-reacting wet bulb/dry bulb sensors. Do not use lithium chloride sensors because of their sensitivity to condensation.

a. When the wet bulb control method is used, clean the wet bulb assembly and install a new wick for each test.

b. In order to produce the evaporation necessary for sensor measurement of wet bulb temperature, ensure the air velocity across the wet bulb is not less than 4.6 m/s (900 ft/min).

c. Because heat from fan motors may affect temperature readings, do not install wet and dry bulb sensors close to the discharge side of any local fan or blower used to create the requirement of paragraph 4.2.1b.

4.2.2 Circulation.

Maintain free circulation of air around the test item and keep the contact area of fixtures supporting the test item to a minimum.

4.2.3 Steam.

Do not inject steam directly into the test chamber working space where it may have an adverse effect on the test item and microbial activity.

4.2.4 Unless Otherwise Specified.

a. Use only reagents that conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

b. Use water as described in Part One, paragraph 5.16. The intent is to not introduce contaminants or acidic/alkaline conditions that may affect the test results.

4.3 Test Interruption.

Test interruptions can result from two or more situations, one being from failure or malfunction of test chambers or associated test laboratory equipment. The second type of test interruption results from failure or malfunction of the test item itself during operational checks

4.3.1 Interruption Due To Chamber Malfunction.

a. General. See Part One, paragraph 5.11, of this Standard.

b. Specific to This Method. The fungus test, unlike other environmental tests, involves living organisms. If the test is interrupted, the fact that live organisms are involved must be considered. Observation of the control test strips should be performed weekly in order to determine corrective action.

(1) If the interruption occurs during the first seven days of the test, restart the test from the beginning with either a new or cleaned test item. If the interruption (such as a short power outage) does not produce drastic drops in humidity (less than 90 percent RH) and temperature (less than 28 °C (82 °F)), continue the test and add at least twelve hours to the final test time.

(2) If the interruption occurs between 8 and 25 days of testing, examine the control strips for evidence of fungus growth. The control strips should be observed weekly (through the glass windows or doors without opening the chamber), if possible. Labs should make a point of hanging at least one additional control in an easily visible location in the chambers. If the controls exhibit viable growth but there is no evidence of fungus growth on the test item, follow the guidance given below.

(3) If the interruption occurs after 25 days of testing, examine the test item for evidence of fungus growth. If the test item is bio-susceptible, there is no need for a retest. If the controls exhibit viable growth but there is no evidence of fungus growth on the test item, follow the guidance given below.

(a) Lowered Temperature. A lowering of the test chamber temperature generally will retard fungus growth. If the relative humidity has been maintained, reestablish the test conditions and continue the test from the point where the temperature fell below the prescribed tolerances. If not, see paragraph 4.3.1.b(3)(c) below.

(b) Elevated Temperature. Elevated temperatures may have a drastic effect on fungus growth. A complete re-initiation of the test may be required if one of the following conditions exist:

(1) The temperature exceeds 40 °C (104 °F).

(2) The temperature exceeds 32 °C (90 °F) for 4 hours or more. A trained microbiologist can determine if the conditions warrant a restart.

(3) There is evidence of deterioration of the fungus growth on the control strips.

Otherwise, reestablish test conditions and continue the test from the point of interruption.

(c) Lowered Humidity. A complete retest may be required if one of the following conditions exist:

(1) The relative humidity drops below 50 percent.

(2) The relative humidity drops below 70 percent for 4 hours or more.

(3) If the relative humidity drops between 70-90 percent for more than 24 hours, restart the test if there is evidence of fungal deterioration on the strips.

(4) There is any evidence of deterioration of the fungal colonies on the control strips. Consider use of newly prepared control strips after any test interruptions to aid in identifying new/continued growth.

Otherwise, reestablish test conditions and continue the test from the point of interruption.

4.3.2 Interruption Due To Test Item Operation Failure.

Failure of the test item(s) to function as required during operational checks during or following testing presents a situation with several possible options.

a. The preferable option is to replace the test item with a "new" one and restart from Step 1.

b. A second option is to replace / repair the failed or non-functioning component or assembly with one that functions as intended, and restart the entire test from Step 1.

4.4 Test Execution.

4.4.1 Cleaning.

Although it is preferable to use a new test item, a used item may be substituted. Cleaning (not sterilizing) can consist of wiping with a damp (water or other solution) cloth or following the test item cleaning instructions. If a solution other than water is used during the cleaning process, document the cleaning solution used in the test report. The cleaning shall be conducted at least 72 hours before test initiation. Prepare the test item in accordance with paragraph 4.5.1.

4.4.2 Miscellaneous.

a. This Method is designed to provide optimal climatic conditions and all of the basic inorganic minerals needed for growth of the fungal species used in the test. The group of fungal species was chosen for its ability to attack a wide variety of materials commonly used in the construction of military materiel. Optional species may be added to the inoculum, if required (see paragraph 2.2.2).

b. This test must be performed by trained personnel at laboratories specially equipped for microbiological work.

c. The presence of moisture is essential for spore germination and growth. Generally, germination and growth will start when the relative humidity of the ambient air exceeds 70 percent. Development will become

progressively more rapid as the humidity rises above this value, reaching a maximum in the 90 to less than 100 percent relative humidity range.

d. The specified temperature of 30 ± 2 °C (86 ± 3.6 °F) is most conducive to the growth of the test fungi.

e. Control items specified in paragraph 4.4.3.3 are designed to:

(1) Verify the viability of the fungus spores used in the inoculum.

(2) Establish the suitability of the chamber environment to support fungus growth.

f. Although this procedure can provide information on the susceptibility of materials to fungus growth, the testing of materials and parts will not reveal potential fungus growth situations in materiel that can result due to the complexities involved in assemblages. Examples are induced conditions created by coatings and protective wrappings, deterioration of protective coatings due to bi-metallic reactions, and other situations that would not be encountered with the testing of components.

4.4.3 Preparation for Test.

4.4.3.1 Preparation for Mineral Salts Solution.

a. Using clean apparatus, prepare the mineral salts solution to contain the following:

Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7 g
Potassium monohydrogen orthophosphate (K_2HPO_4)	0.7 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0.7 g
Ammonium nitrate (NH_4N0_3)	1.0 g
Sodium chloride (NaCl)	0.005 g
Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$)	0.002 g
Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	0.002 g
Manganous sulfate monohydrate ($MnSO_4.H_2O$)	0.001 g
Distilled water	1000 ml

b. Ensure the pH of the mineral salts solution is between 6.0 and 6.5.

c. Sterilize the solution for at least 30 minutes at 121 °C (250 °F) and 15 psi or according to the autoclave manufacturer's recommendations. To avoid precipitation of the solution during heating, separate the (KH_2PO_4) and (K_2HPO_4) from the rest of the solution. After autoclaving, combine the solutions and bring to final volume after cooling. If another sterilization technique is used in lieu of autoclaving (such as filter sterilization), state the procedure used in the test report.

4.4.3.2 Preparation of Mixed Spore Suspension.

> **NOTE - PRECAUTIONS**: Although the exact strains of fungus specified for this test are not normally considered to present a serious hazard to humans, certain people may develop allergies or other reactions. Therefore, use standing operating procedures/good laboratory housekeeping techniques for safety. Also, use only personnel trained in microbiological techniques to conduct the tests.

a. Use aseptic techniques to prepare the spore suspension containing the test fungi determined from paragraph 2.2.2. All hardware used should be sterile (as packaged or autoclaved) and the prep area should be disinfected. A bio-safety cabinet should be used to eliminate cross-contamination of spores or their release to the surrounding laboratory air.

b. Maintain pure cultures of these fungi separately on an appropriate medium such as potato dextrose agar, but culture *Chaetomium globosum* on strips of filter paper overlaid on the surface of mineral salts agar. Prepare the mineral salts agar by dissolving 15.0g of agar in a liter of the mineral salts solution described in paragraph 4.4.3.1.

NOTE: Do not keep the stock cultures for more than 6 months at 6 ± 4 °C (42 ± 7 °F). After that time, prepare subcultures and use them for the new stocks.

c. Visually verify the purity of fungus cultures before the test.

d. Make subcultures from the pure stock cultures and incubate them at 30 ± 2 °C (86 ± 3.6 °F) and greater than 90 but less than 100 percent relative humidity for 10 to 21 days. Most fungi will develop within 10 to 14 days and may show signs of deterioration after longer incubation.

e. Prepare a spore suspension of each of the required test fungus by pouring into one subculture of each fungus 10 ml of a sterilized aqueous solution containing 0.05g per liter of a nontoxic wetting agent such as sodium dioctyl sulfosuccinate or sodium lauryl sulfate. There are several ways to aseptically harvest the necessary quantity of fungal spores. One way is to gently scrape the surface growth from the culture of the test organisms. Pour the spore charge into an appropriately sized sterile, Erlenmeyer flask containing sterilized water and glass beads (10 to 15 glass beads with a diameter of 4mm to 6mm has proven sufficient).

f. Shake the flask vigorously to liberate the spores from the fruiting bodies. Filter as needed using sterile glass wool (i.e., Pyrex 3950 filtering fiber) in a sterile glass funnel to remove mycelium, but leave spores in solution. Centrifuge the filtered spore suspension and discard the supernatant liquid. Wash the pure suspensions with sterile water until the supernatant is clear.

g. Dilute the final washed residue with the sterilized mineral-salts solution in such a manner that the resultant spore suspension contains 1,000,000 ± 20 percent spores per milliliter as determined with a counting chamber.

h. Repeat this operation for each organism used in the test.

i. Perform a viability check for each organism in accordance with paragraph 4.4.3.3.

j. Blend appropriate volumes of the resultant spore suspensions to obtain the final mixed spore suspension.

k. If a different technique is used to harvest the spores, state the procedure in the test report.

NOTE: Use a freshly prepared spore suspension. If not freshly prepared, it should be held at 6 ± 4 °C (42 ± 7 °F) for not more than 14 days.

4.4.3.3 Control Items.

Two types of control tests are required. Using the following procedures, verify the viability of the spore suspension and its preparation, as well as the suitability of the chamber environment.

a. Viability of spore suspension.

(1) Before preparing the composite spore suspension, inoculate sterile potato dextrose (or another nutrient agar) plates with 0.2 to 0.3 ml of the spore suspension of each of the individual fungus species. Use separate agar plates for each species.

(2) Distribute the inoculum over the entire surface of the plate.

(3) Incubate the inoculated potato dextrose agar plates at 30 ± 2 °C (86 ± 3.6 °F) for 7 to 10 days.

(4) After the 7 to 10 day incubation period, check the fungus growth.

NOTE: The absence of copious growth of any of the test organisms over the entire surface in each container will invalidate the results of any tests using these spores.

b. Verifying test chamber environment.

(1) To ensure proper conditions are present in the incubation chamber to promote fungus growth, install control strips into the chamber which have been soaked in the following prepared solution:

Potassium dihydrogen orthophosphate (KH_2PO_4)	0.1 g
Ammonium nitrate (NH_4N0_3)	0.1 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0.025 g
Yeast extract	0.05 g
Distilled water to a total volume of	100 ml
A nontoxic wetting agent such as sodium dioctyl sulfosuccinate or sodium lauryl sulfate	0.005 g
Glycerol	10.0 g

Use HCl and NaOH to adjust the final solution pH to 5.3 (this solution is not sterilized but used immediately in preparation of the control strips).

(2) Prepare control strips from unbleached, plain weave, 100 percent cotton cloth, such as MIL-T-43566, (Type I, Class I only, commercially available). Sterilize the control strips prior to soaking in the above solution. After the strips are thoroughly wetted, remove the excess liquid from the strips and hang them to dry. (Use only strips devoid of fungicides, water repellents and sizing additives. To aid in removing any possible treatment materials, recommend boiling in distilled water (not required if using MIL-T-43566, Type 1, Class I)).

(3) Place the strips vertically within the chamber close to and bracketing the test items to ensure the test strips and test items experience the same test environment The width of the strips should be such that they can be easily viewed for growth during testing (recommend at least 1.9 cm (0.75 inches width).

4.5 Test Procedure.

4.5.1 Preparation for Incubation.

Step 1 Assure the condition of the items to be tested is similar to their condition as delivered by the manufacturer or customer for use, or as otherwise specified. Accomplish any cleaning of the test item at least 72 hours before the beginning of the fungus test to allow for evaporation of volatile materials.

Step 2 Install the test item in the chamber or cabinet on suitable fixtures, and remove any covers. (see note and Step 5 below).

Step 3 Hold the test item in the test chamber at 30 ± 2 °C (86 ± 3.6 °F) and a RH of at least 90 percent but less than 100 percent for at least four hours immediately before inoculation.

Step 4 Inoculate the test item and the cotton fabric chamber control items with the mixed fungus spore suspension by spraying the suspension on the control items and on and into the test item(s) (if not permanently or hermetically sealed) in the form of a fine mist from an atomizer or nebulizer. Ensure personnel with appropriate knowledge of the test item are available to aid in exposing its interior surfaces for inoculation.

> **NOTE**: In spraying the test and control items with composite spore suspension, cover all external and internal surfaces that are exposed during use or maintenance. If the surfaces are non-wetting, spray until drops begin to form on them.

Step 5 In order for air to penetrate, replace the covers of the test items without tightening the fasteners.

Step 6 Start incubation (paragraph 4.5.2) immediately following the inoculation.

4.5.2 Incubation of the Test Item.

Step 1 Except as noted in Step 2 below, incubate the test items at constant temperature and humidity conditions of 30 ± 2 °C (86 ± 3.6 °F) and a relative humidity of at least 90 percent but less than 100 percent for the test duration (28 days, minimum).

Step 2 Inspect the growth on the control cotton strips after 7 days to verify the environmental conditions in the chamber are suitable for growth. Verify that at least 90 percent of the surface area of each test strip located at the level of the test item is covered by fungus (fungus may not have developed color. At 14 days, fungus can be readily seen and 90% coverage of strip should be verified). If it is not, repeat the entire test with the adjustments of the chamber required to produce conditions suitable for growth or check spore viability. Leave the control strips in the chamber for the duration of the test and visually check the growth weekly (observe through window).

Step 3 If the cotton strips show satisfactory fungus growth after 7 days, continue the test for the required period from the time of inoculation as specified in the test plan. If there is no increase in fungus growth on the cotton strips at the second inspection interval (day 14) of the test as compared to the initial 7-day results, the test is invalid and see paragraph 4.3.1for guidance.

Step 4 If the end of designated incubation time falls on a holiday or non-work day due to a scheduling issue or extension of the test due to environmental parameter outliers, extend the test time as needed and note all deviations in the final report.

4.5.3 Inspection.

At the end of the incubation period inspect the test item immediately and if possible, within the chamber with the circulation fans off. If the item is removed from the chamber to conduct the inspection, recommend completing the inspection within 4 hours. If the inspection takes longer than 4 hours, return the item to the chamber or a similar humid environment for a minimum of 2 hours prior to completing inspection. Record the results of the inspection.

4.5.4 Operation/Use.

(To be conducted only if required.) If operation of the test item is required (e.g., electrical materiel), conduct the operation in the inspection period as specified in paragraph 4.5.3. Ensure personnel with appropriate knowledge of the test item are available to aid in exposing its interior surfaces for inspection and in making operation and use decisions. Disturbance of any fungus growth must be kept to a minimum during the operational checkout. If the test item fails to operate as intended, see paragraph 5, and follow the guidance in paragraph 4.3.2.

WARNING: Because of the potential hazardous nature of this test, operation/use by personnel with appropriate knowledge of the test item will be performed under the guidance of technically-qualified personnel (e.g., microbiologists). Appropriate personal protective equipment (PPE) must be worn.

4.6 Decontamination.

See Annex A.

5. ANALYSIS OF RESULTS.

In addition to the guidance provided in Part One, paragraphs 5.14 and 5.17, the following information is provided to assist in the evaluation of the test results. Apply any data relative to failure of a test item to meet the requirements of the materiel specifications to the test analysis, and consider related information such as:

a. Any fungal growth on the test item must be analyzed to determine the species.

b. Any fungal growth on the test item material(s), whether from the inoculum or other sources, must be evaluated by qualified personnel for:

(1) The extent of growth on susceptible components or materials. Use Table 508.8-II as a guide for this evaluation, but any growth must be completely described.

(2) The immediate effect such as discoloration or other visual degradation that the growth has on the physical characteristics of the materiel.

(3) The long-range effect that the growth could have on the materiel (possible storage issues if degradation is noted).

c. Evaluate human factors effects (paragraph 2.1.1.3).

d. Cleaning solution used if other than water.

Table 508.8-II. Evaluation scheme for visible effects.

AMOUNT OF GROWTH	RATING	COMMENTS
None	0	Substrate is devoid of microbial growth.
Trace	1	Scattered, sparse or very restricted microbial growth.
Light	2	Intermittent infestations or loosely spread microbial colonies on substrate surface. Includes continuous filamentous growth extending over the entire surface, but underlying surfaces are still visible.
Medium	3	Substantial amount of microbial growth. Substrate may exhibit visible structural change.
Heavy	4	Massive microbial growth.

6. REFERENCE/RELATED DOCUMENTS.

6.1 Referenced Documents.

a. AR 70-38, Research, Development, Test and Evaluation of Materiel for Extreme Climatic Conditions.

b. STANAG 4370 Environmental Testing.

c. Allied Environmental Conditions and Test Publication (AECTP) 300 (under STANAG 4370), Climatic Environmental Testing, Method 308.

d. MIL-HDBK-454, General Guidelines for Electronic Equipment

6.2 Related Documents.

a. Specifications of the Committee on Analytical Reagents of the American Chemical Society.

b. ASTM 1193 Standard Specification for Reagent Water.

c. Egbert, Herbert W. “The History and Rationale of MIL-STD-810 (Edition 2)”, January 2010; Institute of Environmental Sciences and Technology, Arlington Place One, 2340 S. Arlington Heights Road, Suite 100, Arlington Heights, IL 60005-4516.

(Copies of Department of Defense Specifications, Standards, and Handbooks, and International Standardization Agreements are available online at https://assist.dla.mil.

Requests for other defense-related technical publications may be directed to the Defense Technical Information Center (DTIC), ATTN: DTIC-BR, Suite 0944, 8725 John J. Kingman Road, Fort Belvoir VA 22060-6218, 1-800-225-3842 (Assistance--selection 3, option 2), http://www.dtic.mil/dtic/; and the National Technical Information Service (NTIS), Springfield VA 22161, 1-800-553-NTIS (6847), http://www.ntis.gov/.

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METHOD 508.8, ANNEX A

DECONTAMINATION OF TEST EQUIPMENT AND TEST ITEMS AFTER EXPOSURE TO FUNGUS

1. Decontamination of test equipment, materials, and test items that have been subjected to a fungus test is paramount when the test items are to be sent back to the users, manufacturer, or material management office for further evaluation or reuse. Many test items are too expensive to scrap and must be decontaminated.

2. Decontamination and disinfection of the test chamber.

   a. Initially, good housekeeping procedures should be followed for all testing, especially those tests involving live cultures.

   b. Prior to any testing, the climatic chamber should be thoroughly cleaned inside with a hot, soapy water (or Lysol®-type, parachlorometaxylenol or other microbial decontaminant cleaner) solution.

   c. With no items in chamber, apply high heat (at least 60 °C (140 °F)) and humidity (greater than 90 percent RH) for at least 2 hours. Dry the chamber at 60 °C (140 °F) with no humidity prior to cooling the chamber to ambient. Place the test items in the chamber for fungus testing.

   d. After testing is complete and the items have been examined/pictures taken, the items and the chamber can be initially sterilized with high heat as above and at least 90 percent relative humidity for at least 2 hours. The humidity keeps the surfaces wet until the spores are destroyed.

> **NOTE:** The test items must be able to withstand the high temperature chosen for initial sterilization without damage. Check the test item user´s manual for the storage temperature before proceeding. If the heat is less than 40 °C (104 °F), a longer decontamination time will be needed (up to several days).

   Alternatively, the chamber can be washed with a sodium or calcium hypochlorite solution at 5000 ppm concentration (wear appropriate personal protective equipment (PPE) when using any chemical solutions). A phenolic disinfectant spray can also be used and a Lysol®-type solution will also help control microbial growth. Copious flushing with water to rinse the chamber is needed to limit the chlorine contact on the metal surfaces.

   e. If the test items are washable, follow the instructions for each item and launder in a machine, if possible.

   f. If the items cannot be washed with a solution, wipe with a damp cloth that has been sprayed with a phenolic solution (disinfectant spray) and label the items appropriately with precautions on handling items that have been subjected to fungus testing. Personnel trained in microbiological techniques and who conduct these tests should have general operating procedures in place for handling fungus cultures and test items after exposure.

   g. Perform chamber disinfection after each fungus test. This will ensure a clean test chamber is used, and will help eliminate fungus spores from contaminating the next test. Be sure to disinfect all surfaces and hangers used during testing as well.

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METHOD 508.8, ANNEX B

FUNGUS-INERT MATERIALS

(See paragraph 6.1, reference d)

NOTE: Although the basic (documented) resistance of materials to fungal growth shown below is helpful in the design of new materiel, it is unreliable in determining the fungal susceptibility of complex materials, and the use of testing by analysis is discouraged. The combination of materials, the physical structure of combined materials, and the possible contamination of resistant materials during manufacture is beyond the purview if superficial analysis, and necessitates laboratory or natural environment tests to verify the resistance of the assembled materiel to fungal growth. Caution: The below Table is not a comprehensive list and does not necessarily reflect modern day formulations of materials.

Table 508.8B-I. Fungi susceptibility of materials.

Group I - Fungus-inert materials (Fungus-inert in all modified states and grades)	
Acrylics	Polyamide[1]
Acrylonitrile-styrene	Polycarbonate
Acrylonitrile-vinyl-chloride copolymer	Polyester-glass fiber laminates
Asbestos	Polyethylene, high density (above 0.940)
Ceramics	Polyethylene terephthalate
Chlorinated polyester	Polyimide
Flourinated ethylenepropylene copolymer (FEP)	Polymonochlorotrifluoroethylene
Glass	Polyporpylene
Metals	Polystyrene
Mica	Polysulfone
Plastic laminates:	Polytetrafluoroethylene
Silicone-glass fiber	Polyvinylidene Chloride
Phenolic-nylon fiber	Silicone resin
Diallyl phthalate	Siloxane-polyolefin polymer
Polyacrylonitrile	Siloxane polystyrene
Group II - Fungus nutrient materials (May require treatment to attain fungus resistance)	
ABS (acrylonitrile-butadiene-styrene)	Polydichlorostyrene
Acetal resins	Polyethylene, low & medium density (0.940 and below)
Cellulose acetate	Polymethyl methacrylate
Cellulose acetate butyrate	Polyurethane (ester types are particularly susceptible)
Epoxy-glass fiber laminates	Polyricinoleates
Epoxy-resin	Polyvinyl chloride
Lubricants	Polyvinyl chloride-acetate
Melamine-formaldehyde	Polyvinyl fluoride
Organic polysulphides	Rubber, natural and synthetic
Phenol-formaldehyde	Urea-formaldehyde

NOTE: 1. Literature shows that, under certain conditions, polyamides may be attacked by selective micro-organisms. However, for military applications, they are considered Group I.

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