MILITARY STANDARD

ENVIRONMENTAL TEST METHODS

TO ALL HOLDERS OF MIL-STD-810C:

1. THE FOLLOWING TEST METHOD HAS BEEN REVISED AND SUPERSEDES THE TEST METHOD LISTED:

New Method No.	Date	Supersedes Method No.	Date
508.2	7 April 1981	508.1	10 March 1975

2. RETAIN THIS NOTICE AND INSERT BEFORE TABLE OF CONTENTS.

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Project No. MISC-0C73

FSC MISC

METHOD 508.2

FUNGUS

Section I

I.1 INDEX

I.2 PURPOSE. The purpose of the fungus chamber test is to assess the extent to which the test item will support fungal growth and how the fungal growth may affect performance or use of the test item.

I.3 ENVIRONMENTAL EFFECTS. Fungal growth impairs the functioning or use of equipment by changing the physical properties of the equipment.

I.3.1 Damage mechanisms. The damage mechanisms are as follows:

a. Direct attack on materials. Non-resistant materials are susceptible to direct attack as the fungi break the material down and use it as food. This results in deterioration affecting the physical properties of the material. Examples of non-resistant 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, which contain organic fillers of laminating materials.

(d) Paints and varnishes which 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 which find their way onto equipment during manufacture or accumulate during service can cause 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 fungi, cause corrosion of metals, etching of glass or staining or degrading of plastics and other materials.

(3) the products of fungal growth on adjacent materials, which are susceptible to direct attack, come in contact with the resistant materials.

I.3.2 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. Fungal growth can form undesirable electrical conducting paths across insulating materials 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 fungal growth can adversely affect light transmission through the optical system; block delicate moving parts; and change non-wetting surfaces to wetting surfaces with resulting loss in performance.

I.3.3 Health and aesthetic factors. Fungus growth on equipment can cause physiological problems, (e.g. allergies) or be so aesthetically unpleasant that the users will be reluctant to use the equipment.

I.4 GUIDELINES FOR DETERMINING TEST PROCEDURES AND TEST CONDITIONS. Since microbial deterioration is a function of temperature and humidity and is an inseparable condition of hot-humid tropics and the midlatitudes, it must be considered in the design of all standard general purpose materiel.[1] Method 508.2 (Fungus) consequently is used when an item is to be tested to determine if fungus growth will occur and if so, how it will affect the use of the item.

[1]From AR 70-38, Research, Development, Test and Evaluation of Material for Extreme Climatic Conditions, Chapter 2, Climatic Criteria.

a. Options. The options are limited to extending the test duration and performing a functional test prior to and following the fungus test.

b. The related test conditions involve primarily:

(1) Test time (28 to 84 days).

(2) Test configuration - whether the test item shall be exposed in other than an unsealed condition.

I.4.1 Choice of test phases

a. The operational purpose of the test item. From the requirements, determine the functions to be performed by the equipment and any limiting conditions, (e.g. storage, human factors aspects, whether it is a large volume/low cost/ expendable item, etc.).

b. Required test data. The primary questions that may be addressed after the fungus test are:

(1) Will fungus grow on the item?

(2) How rapidly will it grow on the test item?

(3) How does the fungus growth affect the test item?

(4) To what extent will the fungus affect the mission of the item?

(5) Can the test item be stored effectively in a field environment?

(6) Is the test item safe for use following fungal growth?

(7) Are there simple reversal processes, e.g. wiping off fungal growth?

c. Selection of test phases. Three test phases are included within Method 508.2 (pre-test, chamber and performance). Based on the test requirements document, determine which phases are applicable.

(1) Phase I (pre-test) - Phase I is conducted in all cases to provide baseline data.

(2) Phase II (chamber) - Phase II is the basic fungus test and is conducted in all cases.

(3) Phase III (performance) - Phase III is used to determine compliance with the performance requirements of the test item following the fungus chamber test.

I.4.2 Choice of related test conditions. Recognizing that at least two of the test phases must be conducted, the primary decisions must be made concerning test duration and test item configuration.

a. Test duration. A test duration of 28 days is considered to be the minimum test period. Since indirect effects and physical interference are not likely to occur in the relatively short time frame of the fungus test, extension of the exposure period to 84 days should be considered if a greater degree of certainty (lesser risk) concerning the existence of fungal growth or determining the effect of fungal growth is required.

b. Test item configuration. The test item configuration is an important factor. Even though equipment is to be protected by a container, the container could leak and entrap moisture. As a minimum, the tester should consider the following testing configurations:

(1) In usual shipping/storage container or transit case.

(2) Under realistic storage and use conditions.

(3) With restraints (such as with openings that are normally covered, or commercial equipment not designed for military use).

c. Additional guidelines. Review the equipment specifications and requirements documents. Apply any additional guidelines as necessary.

I.5 SPECIAL CONSIDERATIONS

I.5.1 Failure analysis

a. Any fungi on the test item must be analyzed to determine if the growth is on the test item materials(s) or on contaminants.

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 the component(s) supporting same. Table 508.2-1 can be used as a guide for this evaluation.

(2) The immediate effect that the growth has on the physical characteristics of the test item.

(3) The long range effect that the growth could have on the test item.

(4) The specific material(s) (nutrient) supporting the growth.

c. Fungal growth must not be disturbed during the operational checkout.

d. Human factors effects must be evaluated.

I.5.2 Test interruption. This policy is designed to provide a standard methodology for selecting a course of action in the event of an unscheduled interruption in a test. Any deviation from this policy shall be explained in the test report. Every case of an interrupted test shall be examined individually using the logical decision process provided in this document. 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.

a. If the interruption occurs early in the test, the test should be restarted from the beginning with a new test item or a cleaned item (see II.3.3.1a).

b. If the interruption occurs late in the test cycle, examine the item for evidence of fungal growth. If the test item is biosusceptible, there is no need for a retest. If there is no evidence of fungal growth or if the interruption occurred early follow the guidance as shown below.

(1) Lowered temperature. A lowering of the test chamber temperature generally will retard fungus growth. If there is no evidence of mycological deterioration and the relative humidity has been maintained as in I.5.1b(3), re-establish test conditions and continue the test from the point that the temperature fell below the prescribed tolerances.

(2) Elevated temperature. Elevated temperatures may have a drastic effect on fungus growth. Any rise in temperature above 31°C (88°F) for a period of 4 hours or more shall result in complete re-initiation of the test. Any more moderate risk in temperature without evidence of mycological deterioration, and maintaining of the relative humidity as in I.5.1b(3), re-establish test conditions and continue the test as if no interruption had occurred.

(3) Lowered humidity. Any drop in humidity levels below 90 percent, for a period of 4 hours or more shall result in complete re-initiation of the test. If a more moderate drop in relative humidity occurs that does not result in fungal deterioration, re-establish test conditions and continue the test as if no interruption had occurred.

When re-initiating a test although it is preferable to use a new test item, the same test item may be used if cleaned as specified in II.3.1a. New cotton control strips shall be placed in the test chamber and both the test item and controls will be reinoculated with the test fungi.

I.5.3 Miscellaneous

a. The fungus test is designed to economically obtain data on the biosusceptibility of materiel. It should not be used for testing of basic materials since a variety of other test procedures, including soil burial, pure culture, mixed culture and plate testing are available.

b. Method 508 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 construction of military equipment.

c. The test temperature and humidity cycle selected for this test involves a 5° Celsius drop in temperature to allow moist air to enter the test item (breathing effect) and condense onto or in the internal components, thus representing a natural environment diurnal cycle.

d. The presence of moisture is essential for spore germination and subsequent growth. Generally, germination and growth will commence 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 90-100 percent relative humidity range.

e. The specified temperature range 24-31°C (75-88°F) is most conducive for fungal growth known to cause damage to equipment.

f. Control items specified in phase II are designed to:

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

(2) Establish the suitability of the chamber environment conducive for fungal growth.

g. Testing of equipment for resistance to fungal growth is a specialized technique requiring both appropriate technical knowledge and access to proper fungus cultures and test equipment. Such testing must be preformed at laboratories specially equipped for the work by trained personnel.

I.6 REFERENCES

TABLE 508.2-1 Microbial test evaluation scheme.

Amount of Growth	Percent of Area of Component Covered	Grade	Organic Substrates
None	0	0	Substrate is devoid of microbial growth.
Trace	1-10	1	Sparse or very restricted microbial growth and reproduction. Substrate utilization minor or inhibited. Little or no chemical, physical, or structural change detectable.
Slight	11-30	2	Intermittent infestations or loosely spread microbial colonies on substrate surface and moderate reproduction.
Moderate	31-70	3	Substantial amount of microbial growth and reproduction. Substrate exhibiting chemical, physical or structural change.
Severe	71-100	4	Massive microbial growth or reproduction. Substrate decomposed or rapidly deteriorating.

MIL-STD-810C

METHOD 508.2

FUNGUS

Section II

II.1 APPARATUS. The required apparatus consists of chambers or cabinets, together with auxiliary instrumentation capable of maintaining and monitoring the specific conditions of temperature and humidity and complies with 3.3.1, General Requirements.

II.2 PREPARATION FOR TEST

a. The chamber and accessories shall be constructed and arranged in such a manner as to avoid condensate dripping on the test item.

b. The chamber shall be vented to the atmosphere to prevent the build up of total pressure.

c. Relative humidity shall be determined from the dry bulb - wet bulb temperature comparison method or an equivalent method approved by the procuring activity.

(1) When the wet bulb control method is used, the wet bulb assembly shall be cleaned and a new wick installed for each test.

(2) The air velocity across the wet bulb shall not be less than 4.6 meters per second (900 feet per minute).

(3) The wet and dry bulb sensors shall not be installed in the discharge side of any local fan or blower used to create the requirements of II.2.c(2).

d. Provisions shall be made for controlling the flow of air throughout the internal test chamber space so that the air velocity shall be between 1 and 2 meters per second (197-394 ft/min).

e. Free circulation of air around the test item shall be maintained, and the contact area of fixtures supporting the test item shall be kept to a minimum (see 3.3, General Requirements, Test Facilities and Apparatus).

f. Continuous recordings of test section temperature and relative humidity conditions shall be taken.

g. Readout charts shall be capable of being read with a resolution within 0.6°C (1°F).

h. The desired humidity shall be generated by using steam, or distilled, demineralized or dionized water having a pH value between 6.0 and 7.2 at 23°C (73°F).

(1) Live steam shall not be injected directly into the test section working space where it may have an adverse affect on the test item and microbial activity.

(2) Rust or corrosive contaminants shall not be imposed on the test item by the test facility.

i. Unless otherwise specified:

(1) All reagents shall conform to the specification of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

(2) References to water shall be understood to mean distilled water or water of equal purity.

j. If the test is interrupted, follow the procedure of I.5.1.

II.3 PROCEDURES. The following test phases are provided for application in combinations as required. Prior to initiating any testing, determine:

a. The test duration(s) (I.4.2a).

b. The test item configuration(s) (I.4.2b).

II.3.1 Phase I. Pre-test checkout - All items require a pre-test checkout to provide baseline data. Conduct the checkout as follows:

Step 1. Prepare the test item in accordance with 3.2.2 General Requirements, and required test item configuration (II.3b).

Step 2. Conduct a complete visual examination of the test item with special attention to discolored areas, imperfections, or the existence of any other conditions that could be conducive to fungus growth.

Step 3. Document the results of step 2.

Step 4. Conduct an operational checkout in accordance with the approved test plan if operation is specified by requirements document during or following the fungus test.

Step 5. Record results for compliance with 3.2.2, General Requirements.

Step 6. Proceed to phase II.

II.3.2 Phase II. Chamber test.

II.3.2.1 Test preparation.

II.3.2.1.1 Preparation of mineral salts solution.

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

Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7g
Potassium monohydrogen orthophosphate (K_2HPO_4)	0.7g
Magnesium sulphate heptahydrate ($MgSO_4.7\ H_2O$)	0.7g
Ammonium nitrate (NH_4NO_3)	1.0g
Sodium chloride (NaCl)	0.005g
Ferrous sulfate heptahydrate ($FeSO_4.7\ H_2O$)	0.002g
Zinc sulfate heptahydrate ($ZnSO_4.7\ H_2O$)	0.002g
Manganous sulfate monohydrate ($MnSO_4.\ H_2O$)	0.001g
Distilled water	1000ml

b. Measure the pH of the mineral salts solution. If not between 6.0 and 6.5, discard and prepare a proper solution.

II.3.2.1.2 Preparation of mixed spore suspension. NOTE: PRECAUTIONS: Although the fungi specified for this test are not normally considered to present a serious hazard to humans, certain people may develop allergies or other reactions. It is therefore recommended that standard operation procedures (SOPs) for safety be employed. It is also recommended that the tests be conducted by personnel trained in microbiological techniques.

a. Using asceptic techniques, prepare the spore suspension containing the following test fungi:

	FUNGUS SOURCE IDENTIFICATION NO.	
FUNGI	USDA*	ATCC**
Aspergillus niger	QM 386	ATCC 9642
Aspergillus flavus	QM 380	ATCC 9643
Aspergillus versicolor	QM 432	ATCC 11730
Penicillium funiculosum	QM 474	ATCC 11797
Chaetomium globosum	QM 459	ATCC 6205

*U. S. Department of Agriculture (SEA/FR)
Northern Regional Research Center
ARS Culture Collection
1815 N. University Street
Peoria, Illinois 60604

(The fungi may be distributed in a lyophilized state, or on agar slants).

**American Type Culture Collection
12301 Parklawn Drive
Rockville, Maryland 20852

b. Maintain pure cultures of these fungi separately on an appropriate medium such as potato dextrose agar except that chaetomium globosum shall be cultured on strips of filter paper overlayed on the surface of mineral salts agar.

c. Prepare mineral salts agar by dissolving 15.0g agar in a liter of the mineral salts solution described in II.3.2.1.1. Note: Do not keep the stock cultures for more than 4 months at 6° ± 4°C (43° ± 7°F); after that time, prepare subcultures and use them for the new stocks.

d. Verify the purity of fungus cultures prior to the test.

e. Incubate subcultures used for preparing new stock cultures or the spore suspension at 30° ± 1.4°C (86° ± 2.5°F) for 14 to 21 days.

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

g. Use a rounded glass rod to gently scrape the surface growth from the culture of the test organism.

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

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

j. Filter the dispersed fungal spore suspension into a flask through a 6 mm layer of glass wool contained in a glass funnel. Note: This process should remove large mycelial fragments and clumps of agar.

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

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

m. Dilute the final washed residue with 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.

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

o. Perform a viability check for each organism in accordance with II.3.2.1.3a.

p. Blend equal volumes of the resultant spore suspension to obtain the final mixed spore suspension. Note: The spore suspension may be prepared fresh. If not freshly prepared, it should be held at 6° ± 4°C (43° ± 7°F) for not more than 7 days.

II.3.2.1.3 Control items. Two types of control tests are required. Using procedure of II.3.3.1.3a verify the viability of the spore suspension and its preparation. Using the procedure of II.3.2.1.3b verify the suitability of the chamber environment.

a. Viability of spore suspension.

(1) Prior to preparing the composite spore suspension inoculate sterile potato dextrose agar plates with 0.2 to 0.3 milliliters of the spore suspension of each of the individual fungal species using separate potato dextrose agar plates for each species.

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

(3) Incubate the inoculated potato dextrose agar plate at 24° to 31°C (75° to 88°F) for 7 to 10 days.

(4) After the incubation period, check the fungal 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. Test chamber environment.

(1) Inoculate a known susceptible substrate along with the test item to insure that proper conditions are present in the incubation chamber to promote fungal growth. Note: The controlled substrate shall consist of cotton fabric strips conforming to MIL-T-43566A Tape, Textile, Cotton, General Purpose, Natural or in Colors, Type 1a, Class 2, bleached, white flat construction.

(2) Prepare the following solution:

(a) 10.0 grams glycerol

(b) 0.1 potassium dihydrogen orthophosphate (KH_2PO_4)

(c) 0.1g ammonium nitrate (NH_4NO_3)

(d) 0.025g magnesium sulfate ($MgSO_4.7\ H_2$)

(e) 0.05g yeast extract

(f) Distilled water to a total volume of 100 ml

(g) HCl or NaOH to adjust the final solution pH to 5.3.

(3) Dip the cotton strips into the above solution. After dipping, remove the excess liquid from the strips and hang them to dry before placing them in the chamber and inoculating them.

(4) Within the chamber, place the strips vertically in close proximity to and bracketing the test item so that the test strips and test items experience the same test environment. The length of the strips shall be at least the height of the test item.

II.3.3 Phase III. Test performance

II.3.3.1 Preparation for incubation

a. Assure that the condition of the test items subjected to testing is similar to that as delivered by the manufacturer or customer for use, or as otherwise specified. Any cleaning of the test item shall be accomplished at least 72 hours prior to the beginning of the fungus test.

b. Install the test item in the chamber or cabinet on suitable fixtures or suspended from hangers.

c. Hold the test item in the operating chamber for at least 4 hours immediately prior to inoculation.

d. Inoculate the test item and cotton fabric chamber control items (II.3.2.1.3b(1) with the mixed fungal spore suspension (II.3.2.1.2) by spraying it on the control and on and into the test item(s) (if not hermetically sealed)[1] in the form of a fine mist from an atomizer or nebulizer. Note: In spraying the test and control items with composite spore suspension care should be taken to cover all external and internal surfaces which are exposed during use or maintenance. If the surfaces are nonwetting, spray until initiation of droplet coalescence.

e. Replace covers of the test items loosely.

f. Start incubation immediately following the inoculation.

II.3.3.2 Incubation of the test item.

a. Incubate the test item(s) under a daily cycle of temperature and humidity conditions consisting of 20 hours of a relative humidity of 95 $\pm$ 5 percent at an air temperature of 30° $\pm$ 1°C (86° $\pm$ 2°F) followed by a 4-hour period in which conditions of 95^{+5}_{-0} percent relative humidity at 25° $\pm$ 1°C (77° $\pm$ 2°F) are maintained for at least 2 hours. Up to a total of 2 hours of the 4-hour period will be used for the transition(s) of temperature and relative humidity. Temperature and humidity conditions during the transition periods must be as follows: temperature 24° to 31°C (75° to 88°F) and relative humidity above 90 percent.

b. Repeat the 24-hour daily cycle for the test duration.

c. After 7 days, inspect the growth on the control cotton strips to assure that the environmental conditions in the chamber are suitable for growth.

[1]Personnel with appropriate knowledge of the test item should be available to aid in exposing its interior surfaces for inoculation.

For this assurance, at least 90 percent of the part of the surface area of each test strip located at the level of the test item should be covered by fungi when inspected visually. If not, repeat the entire test with the required adjustments of the chamber to produce conditions suitable for growth. Leave the control strips in the chamber for the duration of the test; note their condition at this time and record it with the test item data as described in II.3.1, step 3.

d. If the cotton strips show satisfactory fungus growth after 7 days, continue the test for the required period of days from the time of inoculation (I.4.2a). If there is a decrease in fungal growth on the cotton strips at the end of the test as compared to the 7-day results, the test is invalid.

II.3.3.3 Inspection. At the end of the incubation period, inspect the test item immediately. If possible, inspect the item within the chamber. If the inspection is conducted outside of the chamber and not completed in 8 hours, return the test item to the test chamber or similar humid environment for a minimum of 12 hours. Except for hermetically sealed equipment, open the equipment enclosure and examine both the interior and exterior of the test item. Record results of the inspection to include that information of II.4 as applicable. Note: Data shall be used for comparison with that obtained in II.3.1.

II.3.4 Phase III. Operation/usage (to be conducted only if required).

II.3.4.1 If operation is required (e.g. electrical equipment), conduct the operation in the period as specified in II.3.3.3. Data shall be recorded for comparison with the baseline data obtained in II.3.1, step 3. Personnel with appropriate knowledge should be available to aid in exposing interior surfaces of the item for inspection and making operational and use decisions.

II.4 INFORMATION TO BE RECORDED

a. Presence or evidence of fungal growth.

b. Location of fungus.

c. Narrative description of growth, including colors, area covered, growth patterns, density of growth, and thickness of growth.

d. Test period.

e. Effect of fungus on performance or use.

(1) As received from chamber

(2) After use maintenance.

f. Test conditions.

g. Condition of test item at time of test.

h. All deviations from specified test conditions.

i. Whether directly from manufacturer.

j. Test item history (prior tests).

k. Physiological or aesthetic considerations.

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