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NAVAL SURFACE WARFARE CENTER
CARDEROCK DIVISION

NAVAL SHIP SYSTEMS
ENGINEERING STATION
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IN REPLY REFER TO

9504
Ser 965/001
26 January 2007

From: Commanding Officer, Naval Surface Warfare Center,
Carderock Division, Naval Ship Systems Engineering Station
To: Defense Logistics Agency,
Defense Supply Center Columbus (Code VQP)

Subj: FUNGUS RESISTANCE TEST GUIDE, QUALIFIED PRODUCTS LIST, TEST SUITABILITY FOR FIBER OPTIC CABLE TOPOLOGY COMPONENTS

Encl: (1) Fungus Resistance, original release, dated 26 January 2007

1. Purpose.

This letter addresses the requirements for performing the fungus resistance test per MIL-STD-810 on Fiber Optic Cable Topology (FOCT) components. Proper documentation and performance are required for the following: test laboratory suitability status audits from the Defense Supply Center Columbus (DSCC); proper test performance to FOCT military specifications (such as Qualified Products List or QPL inspections); and review of test procedures and test reports. The applicable FOCT military specifications that are under DSCC cognizance and require QPL testing are as follows: MIL-PRF-24623, MIL-I-24728, MIL-PRF-28876, MIL-PRF-29504, MIL-C-83522, MIL-DTL-38999, MIL-PRF-85045. A fungus resistance test guide, enclosure (1) of this letter, has been prepared to address military requirements and methods of performance.

2. Background.

Naval Surface Warfare Center, Carderock Division, Ship Systems Engineering Station (NSWCCD-SSES) is tasked by the Naval Sea Systems Command (NAVSEA) to provide technical support for qualification and test efforts regarding FOCT components. One subtask is to provide technical support/consultation to DSCC. As part of the subtask, NSWCCD-SSES has supported DSCC in past efforts to qualify component vendors. These efforts include auditing their in-house test facilities, auditing independent, commercial test laboratories, clarifying requirements in military specifications, and reviewing documentation (such as test procedures and reports). Development of this fungus resistance test guide is another type of support being provided.

3. Distribution statement

Distribution Statement A: Approved For Public Release, Distribution Is Unlimited.

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4. Addressees.

This letter is intended for DSCC and other Government agencies/activities, parties in direct support of the Government agencies/activities, vendors, and out-of-house (outside the component's vendor facilities or independent) test laboratories.

5. Point of contact.

DSCC-VQP is to be the initial point of contact for the qualification issues/inquiries that pertain to this matter. Principle point of contact is J. Casto. He can be contacted by telephone: (614) 692-7076 or E-mail: john.casto@dla.mil. Alternative point of contact is Richard Marbais. He can be contacted by telephone: (614) 692-0620 or E-mail: richard.marbais@dla.mil. NSWCCD-SSES point of contact for technical support to DSCC on this matter is E. Bluebond.

S. Walicki

S. WALICKI
By direction

Copy to:
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NAVAIR 4.4.4.3 (J. Collins)
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Fungus resistance.

1. Intent. The intent of providing further clarification to this test is to ensure testing is done in a consistent manner. Former testing has shown inconsistent results. Suspected causes are contamination (test sample, stock cultures and testing apparatus), use of different preparations (solutions, mediums, etc.) or procedures and different evaluation criteria. The content below provides further direction for decontamination, processes and evaluation criteria.

2. Requirement (3.8.4). Polymeric cable materials shall show sparse or very restricted microbial growth and reproduction with minor or inhibited substrate utilization. There shall be little or no chemical, physical, or structural change detectable.

3. Test method (4.8.4). Fiber optic components composed of materials not listed as fungus inert in guideline 4 of MIL-HDBK-454 shall be tested in accordance with TIA/EIA-455-56. In addition, the following requirements for decontamination, fungus cultures and evaluation criteria apply.

a. Test criteria.

Test (incubation) duration. Unless otherwise specified, test shall be performed for 28 days.
Test temperature: 86 ± 2 °F (30 ± 1 °C).
Test relative humidity: %RH greater than 90 but less than 100.
Fungi types. Aspergillus flavus (USDA NRRL # 3537, ATCC # 9643),
Aspergillus versicolor (USDA NRRL # 20734, ATCC # 11730),
Penicillium funiculosum (USDA listed as Penicillium funiculosum Thom, USDA NRRL # 3647, ATCC # 11797),
Chaetomium globosum (USDA listed as Chaetomium globosum Kunze: Fries, USDA NRRL # 1870, ATCC # 6205),
Aspergillus niger (USDA listed as Aspergillus niger van Tieghem, USDA NRRL # 3536, ATCC # 9642).

b. Suitability of fungus cultures.

Culture stock acceptable sources of supply. Fungus cultures shall be obtained from either the United States Department of Agriculture (USDA) or the American Type Culture Collection (ATCC). Curator of the Aspergillus and Penicillium collection at theUSDA Microbial Genomics and Bioprocessing Research Unit is Dr. Stephen Peterson (peterssw@mail.ncaur.usda.gov). Web Site for USDA culture collection catalog is http://nrrl.ncaur.usda.gov.
Culture stock replenishment interval. Stock cultures must be replaced after 4 months unless subcultures are actively grown.
Subcultures from pure stock. Stocks of each fungus may be maintained by actively growing them from stock cultures obtained originally from USDA or ATCC. To ensure purity of the actively growing stock cultures, the stock must be renewed every 4 years or less. Risk of contamination occurs each time the stock culture is transferred to prepare the subcultures. Stock cultures shall not be kept more than 4 months at 43 ± 7 °F (6 ± 4 °C). After four months at 43 ± 7 °F, new subcultures must be prepared and used for the new stock.

c. Test sample considerations.

Sample size. Three test samples of each polymeric component or part.
Preparation of cable samples. A 12 inch length of cable shall be tested, strip back 8 inches from the end to expose all cable components. Be advised that it may not be able to determine if there is fungus growth on water blocking material.

d. Pre-test decontamination processes.

Pre-test decontamination of test samples. Test samples are to be decontaminated prior to testing to ensure no organic contamination has occurred. The decontamination procedure and test sample handling after decontamination must be specified in the test procedure. Test samples are to be decontaminated with reagent grade (> 99 percent pure) isopropyl alcohol prior to testing. Other decontamination methods/agents may be used if approved by the Qualifying Activity. Appropriate handling measures are to be taken to ensure recontamination does not occur (such as wearing un-powdered gloves during the decontamination process to ensure no contamination from hand oils). Decontamination shall be done at least 72 hours before the start of the test to ensure that volatile materials have evaporated.
Pre-test decontamination of test apparatus. Decontamination shall be done as cited in annex A of MIL-STD-810, Method 508. Wipe or mop the interior of the chamber with hot water to remove any dirt and dead growth. With no test samples in the environmental chamber, heat the chamber to 140 °F (60 °C) or higher for at least 2 hours (with no humidity). Cool the chamber, pans, etc. to ambient prior to any test sample placement. Alternate procedure for decontamination of containers/pans may be by sterilization in an autoclave at 250 °F (121 °C) or higher and at 15 atmospheres or greater for 20 minutes or more.
Pre-test decontamination of containers. Decontamination of glassware/containment apparatus to prepare the fungus suspension, nutrient solution and test sample containers (with nutrient solution and one of five fungi suspensions) shall be sterilized in an autoclave at 250 °F (121 °C) or higher and at 15 atmospheres or greater for 20 minutes or more.
Purity of water and chemicals used for test. Water shall be distilled or of equal purity. Chemicals used for fungus suspension shall be of reagent grade (as defined by the American Chemical Society).

e. Test processes.

Environmental chamber in which the test is performed shall meet the following criteria:

Temperature range: 82 °F (28 °C) to 140 °F (60 °C) minimum.

Temperature variance: ± 1 °F (± 2 °C).

Relative humidity range: 50 to 98 % RH.

Relative Humidity variance: ± 5 % RH.

Humidity sensor type: solid state type sensor not affected by water condensation (no lithium chloride sensors).

Humidity sensor accuracy: ± 3 % RH.

Temperature sensor accuracy: ± 2 °F (± 1 °C).

Deflectors or screens installed around test item if in air flow stream of chamber air circulation.

Humidity generation: No steam injected directly in portion of test chamber interior where it may adversely affect test item and microbial activity.

Maintaining stock cultures. Each fungus shall be maintained separately on an appropriate medium such as potato dextrose agar. Chaetomium globosum shall be maintained on strips of filter paper overlaid on the surface of mineral salt agar (see 4.4.3.1 and 4.4.3.2b of MIL-STD-810, Method 508).

Spore suspension preparation. Prepare a spore suspension using the process in 4.4.3.2 of MIL-STD-810, Method 508. Note that last step in this process is a blended spore suspension. The positive control for stock culture purity requires a separate spore suspension be prepared for each fungus. Perform this positive control prior to blending.

Spore suspension concentration. Spore suspensions from each individual fungus shall be tested with a counting chamber to verify that the resultant spore suspension contains 1,000,000 ± 200,000 spores per milliliter. A counting chamber or haemocytometer is a microscope slide with squares etched on the slide. With the liquid at a specified depth, the number of spores per square is counted.

Positive control for stock culture purity (viability control). Verify purity of spore suspension by inoculating sterile potato dextrose or another nutrient agar plates with 0.2 to 0.3 ml of the spore suspension of each fungus (i.e. the spore suspensions of each fungus separately prior to blending) and incubate for 7 to 10 days at 86 ± 2 °F (30 ± 1 °C). After this period, the absence of copious growth over the entire surface will invalidate the results of any test using these spores.

Positive control during testing. Prepare control strips from unbleached, plane weave 100% cotton (no fungicides, water repellents or additives) cloth, dip in solution, dry and inoculate with blended spore suspension as specified in 4.4.3.3.b of MIL-STD-810, Method 508.

(1) Control strip dimensions. Control strips should be about 3 cm wide. The length should be at least as long as the tallest test item is high.

(2) Placement. Place the control strips within the environmental chamber hung vertically and bracketing the items being tested.

(3) Control strip inspection after 7 days growth. After 7 days of testing, inspect the growth of the control strips. At least 90 percent of the surface area of each test strip located at the level of the test items should be covered by fungus.

(4) Control strip inspection after 28 days. If there is no increase in the fungus growth at the end of the test as compared to the 7 day period, the test is invalid.

Placement of test items in environmental chamber. Place on suitable fixtures or suspend from hangers. Components may be placed on pans or shallow containers. Ensure adequate air flow around the test samples.

Inoculation (4.5 of MIL-STD-810, Method 508). Run the environmental chamber with test items and control strips inside for 4 hours at test conditions before inoculating the test items and control strips. Test items and control strips are to be inoculated with the blended spore suspension in the form of a fine mist from an atomizer or nebulizer. All external and exposed internal surfaces are to be inoculated. If the surfaces are non-wetting, then spray surfaces until drops begin to form on them.

Incubation period. See 3.a above. Monitor temperature and humidity during the test.

f. Post test decontamination processes.

Post-test decontamination of test samples. Test samples are to be decontaminated after testing and the evaluation performed to ensure no fungal contamination has remained. Decontamination shall be done as cited in annex A of MIL-STD-810. Test samples are to be heated to 140 °F (60 °C) or higher for at least 2 hours and at least 90 percent relative humidity. After this heat sterilization, wash (soak) test samples in a disinfectant (such as heavy chlorine) solution (at 5000 ppm concentration). Place test samples in a plastic bag and label as being subjected to a fungus test. . As an alternative, decontamination may be sterilized in an autoclave at 250 °F (121 °C) or higher and at 15 atmospheres or greater for 20 minutes or more.

Post-test decontamination of test apparatus. Environmental chamber, pans, etc. are to be decontaminated after testing and the evaluation performed to ensure no fungal contamination has remained. Decontamination shall be done as cited in annex A of MIL-STD-810, Method 508. Environmental chamber and other test apparatus are to be heated to 140 °F (60 °C) or higher for at least 2 hours and at least 90 percent relative humidity. After this heat sterilization, wash the environmental chamber, etc. with a sodium or calcium hypochlorite solution at 5000 ppm concentration. Flush chamber interior with water to limit chlorine contact on metal surfaces. As an alternative, decontamination of other apparatus (glassware/containment apparatus) may be sterilized in an autoclave at 250 °F (121 °C) or higher and at 15 atmospheres or greater for 20 minutes or more.

g. Personnel protection. Appropriate personal protective equipment (PPE) must be worn while handling fungus cultures and chemical disinfectant solutions. If ventilation hood with HEPA (high efficiency particulate air) filters (or other suitable airway protection) are not available during spore suspension and test sample inoculation, inspection personnel, at a minimum, should wear goggles and a M95 protective mask (or one sufficient to filter spore particles and bacteria). Refer to safety documentation for specific fungus hazards and Material Safety Data Sheets (MSDS) for chemical hazards. Also, ensure that the chamber is off prior to opening the chamber door. This action also turns off the chamber fans which reduces the spore suspension in the air.

4. Fungus Test Evaluation Criteria. A fungus test evaluation criteria shall be specified in the test procedure. The evaluation criteria listed below may be used or an alternative one proposed. Verify results under magnification to confirm raised fungal growth as opposed to dried residue (see Microscopic Photographs for the Five Fungi on page 5).

a. Rating criteria.

(1) Grade. Pass/fail criteria is based on grade assigned to the observed fungal growth. Grades 0 and 1 meet the fungus resistance requirement. Grades 2 through 4 do not.

	Amount Of Observed Growth On Surface	
Grade	**Keyword**	**Description**
0	None	Devoid of microbial growth. Surface exhibiting no chemical, physical or structural change.
1	Trace	Scattered. sparse or very restricted microbial growth. Appearance on surface minor or inhibited. Surface exhibiting no chemical, physical or structural change.
2	Slight (Light)	Intermittent infestations. Loosely spread microbial colonies on surface/moderate growth. Includes continuous filamentous growth extending over the entire surface. Surface exhibiting no chemical, physical or structural change.
3	Moderate (Medium)	Substantial amount of microbial growth. Surface exhibiting chemical, physical or structural change.
4	Severe (Heavy)	Massive microbial growth. Surface decomposed or rapidly deteriorating.

(2) Supporting description of growth over a specified percentage of the surface in which the microbial growth is observed.

S = Spotty.
P = Patchy.
U = Uniform growth spread over one or more areas, at least one area exceeding 20 percent of surface.

(3) Rating designation. The amount of microbial growth observed is recorded by grade, supporting description and percentage of surface in which the microbial growth is observed.

Example: 1P 20% represents sparse growth that appears in patches over 20 percent of the test sample surface.

(4) Rater's evaluation and contact information. Most raters have considered the pass/fail boundary (showing sparse or very restricted microbial growth and reproduction with minor or inhibited substrate utilization) around 1P for a certain percentage of surface coverage. Since the rater's determination is somewhat subjective, it may be prudent for a Government representative to make contact and discuss test results. The rater's contact information should be included in the test report. The test procedure, or other laboratory documentation, should advise of this request.

b. Mold (mould) identification.

(1) Visual inspection (macroscopic morphology). Observation is mainly by texture and color.
Texture: Woolly to cottony to somewhat granular.
Color:
Aspergillus flavus: Mostly olive to lime green. Droplets of fluid (exudate) may be clear to pale brown. Filaments (hyphae) are brown.
Aspergillus niger. White, quickly turning black.
Aspergillus versicolor. Range from very pale green, to greenish-beige, pinkish-green, salmon green or dark green. Droplets (exudate) from pink to reddish-brown.
Chaetomium spp. White, turning grey to olive.
Pencillium spp. White, turning blue green, gray, green, olive gray, yellow or pinkish.

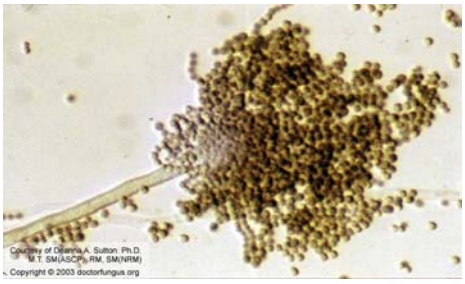
(2) Microscopic inspection (microscopic morphology). Microscopic inspection should be done under 400x or greater. At a minimum, 100x can be used if the features for a typical mould can be observed. A typical mold can be identified by observing hyphae and conidia.
Hyphae are interwoven filaments. These filaments form masses (called mycelium).
Conidia or spores are rounded forms found at the ends of some hyphae.
Photographs for the five fungi used in the fungus resistance test are shown below.

(3) Situations requiring microscopic inspection. Verify change observed is fungus growth when material may change under test conditions (such as gelling of water blocking materials under humid conditions). Verify change observed is fungus growth when uncertainty is due to scarcity of growth or unusual characteristics (such as texture, color or pattern). List magnification used for microscopic inspections.

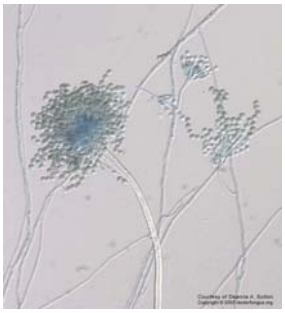
(4) Terminology. Fungi are placed in two groups of yeasts and moulds. The five fungi used in the fungus resistance test are molds. Either spelling of “mould” or “mold” can be used. Mould comes from the Norse word “mowlds” for fuzzy. Mold comes from the French word “molde” for fom or shape. Moulds will appear “fuzzy” with moderate to heavy growth. The word “mold” is more commonly used in the United States to describe this group of fungi, “mould” in the United Kingdom.

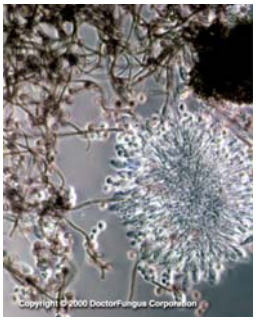
(5) Spore suspension. Suspension that is sprayed to inoculate test samples and positive controls consist almost entirely of spores. Most of the filaments remain anchored within the agar matrix in which a mold is grown. Other filaments are filtered out during spore suspension preparation.

Microscopic Photographs for the Five Fungi

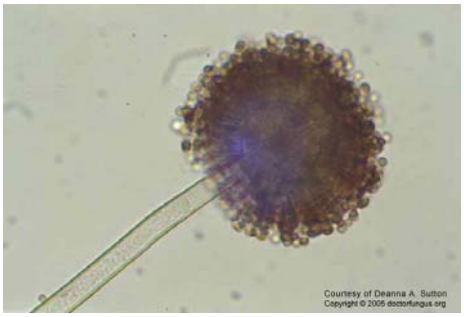


Aspergillus flavus

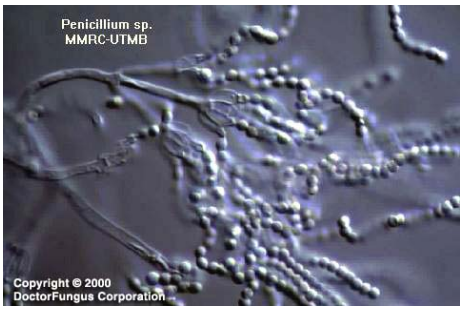

Aspergillus niger.



Aspergillus versicolor



Chaetomium spp.



Pencillium spp.

SAMPLE

DATE: _____ REVIEWED BY: _____
ITEM CODE: _____ APPROVED BY:_____

ITEM DESCRIPTION: ___Fiber Optic Cable – Test Sample # 1___

COMPONENT	GRADE	% AREA	PASS/FAIL
Outer jacket (multiple fiber cable)			
Kevlar (arimid yarn)			
Water Blocking Material			
Central strength member			
OFCC (jacket on single fiber cable)			
900 micron buffer			

Grade:

0 = None. Surface (substrate) is devoid of microbial growth.
1 = Trace. Scattered, sparse or very restricted microbial growth is observed. Appearance on surface is minor or inhibited.
2 = Slight (light). Intermittent infestations or loosely spread microbial colonies on surface/moderate growth.
3 & 4 = Moderate (medium) and Severe (heavy), respectively, exhibit chemical, physical or structural change on surface, Grades 0, 1 and 2 do not for rating purpose.
(3 = Substantial growth. Surface exhibiting chemical, physical or structural change, 4 = Massive growth. Surface decomposed or rapidly deteriorating.)

Specific details of growth:

P = Patchy growth.
S = Spotty microbial growth (colonization or infestation).
U = Uniform growth over the specified percentage of surface area(s), where at least one area exceeds 20 percent of the surface.

SAMPLE

SAMPLE

DATE: _____ REVIEWED BY: _____
ITEM CODE: _____ APPROVED BY: _____

COMPONENT: _____Fiber Optic Connector_____

Component	Sample 1			Sample 2			Sample 3		
	Grade	% Area	P/F	Grade	% Area	P/F	Grade	% Area	P/F
Boot, black									
Plastic boot insert									
Boot, blue									
Plastic boot insert									
Dust cover, blue									
Dust cover, black									
Adapter end cap, blue									
Adapter end cap, black									

Grade:

0 = None. Surface (substrate) is devoid of microbial growth.
1 = Trace. Scattered, sparse or very restricted microbial growth is observed. Appearance on surface is minor or inhibited.
2 = Slight (light). Intermittent infestations or loosely spread microbial colonies on surface/moderate growth.
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SAMPLE