Studies on validity of mildew resistance test for filter media

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Manufacturers no longer need to add fungicides to HEPA filter media to pass the military specifications for mildew resistance. The applicable test method has been modified to provide valid, meaningful results.

Military specifications regarding mildew resistance of fire-resistant, high-efficiency filters and filter media (MIL-F51079D, 4.2.44, March 14, 1985) stipulate that "test of the medium shall be conducted in accordance with Federal Test Method 5750, No. 191."

When high efficiency particulate air (HEPA) filter media produced from glass microfibers repeatedly failed this test, questions arose regarding the appropriateness of the chosen test method for use in evaluating these filter media. The fungus used in the test, *Chaetomium globosum*, is used in tests designed to determine mildew resistance in textiles, not glass fibers. The reason for this is that *C. globosum* requires cellulose for growth on minimal media. Consequently, a study was undertaken to determine why the filter media were failing the test.

Materials, methods, and results

Organisms used and culture procedures

The study was carried out using the test organism specified in the test procedure. The test organism was the strain of *C. globosum* deposited at the American Type Culture Collection (ATCC No. 6205). Routine stock cultures were maintained on V-8 juice agar (ATCC Culture Medium No. 329) because it supports abundant growth of *C. globosum* and is easy to prepare. Stock cultures for all tests were maintained on the prescribed minimal-salts agar (KH_2PO_4, 1.0 g; NH_4NO_3, 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; agar, 20.0 g; distilled H_2O, 1 L). The surface, when hardened, was covered with a 70-mm disc of Whatman No. 2 filter paper.

Test procedures

All tests were run in triplicate and repeated three times. Initially, all media samples were subjected to the mildew-resistance test exactly as prescribed in Federal Test Method 5750, No. 191. This procedure stipulates the following procedures.

Inoculum. The spores of *C. globosum* are born in dark gray to black perithecia (fruiting bodies) and do not readily go into suspension without disruption of the perithecia. This is easily accomplished by scraping the perithecia from the surface of a mature (10–14 days) culture and transferring them to a screw-capped flask or bottle containing about 50 mL of water and an equal volume of 3–6-mm glass beads. Several minutes of vigorous shaking will release the spores from the perithecia, and the resulting suspension can be decanted for use.

Inoculation. The specimen is dipped in a fresh inoculum suspension and placed in contact with the hardened agar in the culture vessel. Other equally satisfactory methods of inoculation include pipetting or spraying.

Incubation. The inoculated specimen is incubated for 14 days at a temperature of 28°C to 30°C (82°F to 86°F).

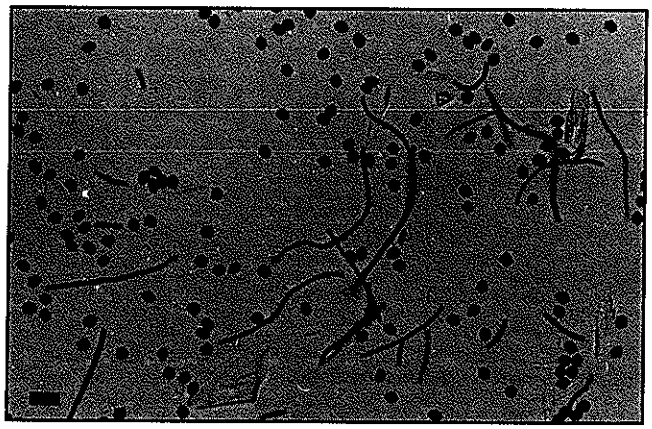
All samples of filter media failed this test. We then attempted to determine the cause of the failure by subjecting individual components of the media to the same test. All of the components failed the test also. At this point, it became apparent that the test procedure itself might be suspect.

As an initial check of the test procedure, the inoculum was placed on minimal agar minus the filter paper. The resultant growth was sufficient for the agar itself to fail the test. It was deduced that the inoculum must contain sufficient cellulose fiber carry-over from the stock culture to promote growth. The inoculum was examined with a microscope. The result, illustrated in **Fig. 1**, indicated that there probably was enough cellulose to support growth of the inoculum.

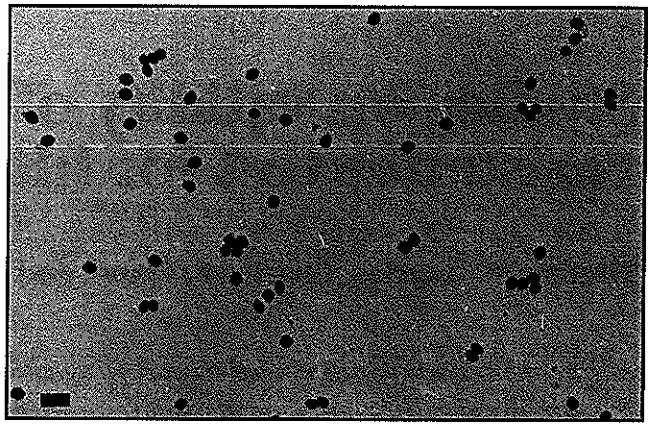
To test this possibility, the inoculum was filtered through a sterile, 2.54-cm-thick layer of glass wool in a Buchner funnel. This procedure effectively removed the fibrillar material, as seen in **Fig. 2**. **Figure 3** shows that the filtered inoculum produced typical, abundant growth of *C. globosum* on the control medium, cellulosic filter paper. **Figure 4** shows the results obtained on filter media produced from glass microfibers for (a) filtered inoculum

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1. Unfiltered spore inoculum showing cellulosic fibers. Bar = 10 μm.



2. Filtered spore inoculum. Note absence of cellulosic fibers. Bar = 10 μm.



and (b) unfiltered inoculum. Both inocula were obtained from the same batch. The unfiltered inoculum produced limited growth on the filter media, just as it had in the previous tests. However, the filtered inoculum produced no growth on filter media, which did not contain any fungicide.

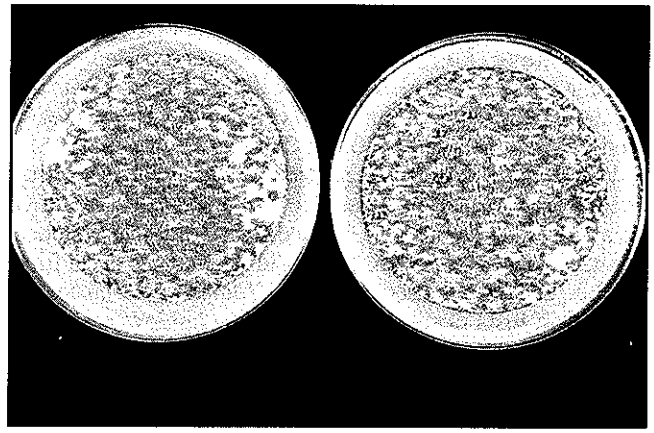
Discussion

The results of this study indicate that Federal Test Method 5750, No. 191, is not appropriate for the purpose for which it was intended in military specification MIL-F-51079D. Both the test procedure and the species of fungus used in the test are inappropriate. While the cellulolytic properties of *C. globosum* make it a prime candidate for testing mildew resistance in textiles, this property has no bearing on its application in testing filter media composed of glass microfibers. A possible exception might arise in an application where a glass-fiber filter was exposed to cellulose fibers. However, under moist conditions, almost any substrate can support fungal growth (mildew) or serve as a carrier for contaminants capable of supporting such growth. Thus fiber media should be tested not only for their ability to sustain fungi. They also should be tested under conditions of use.

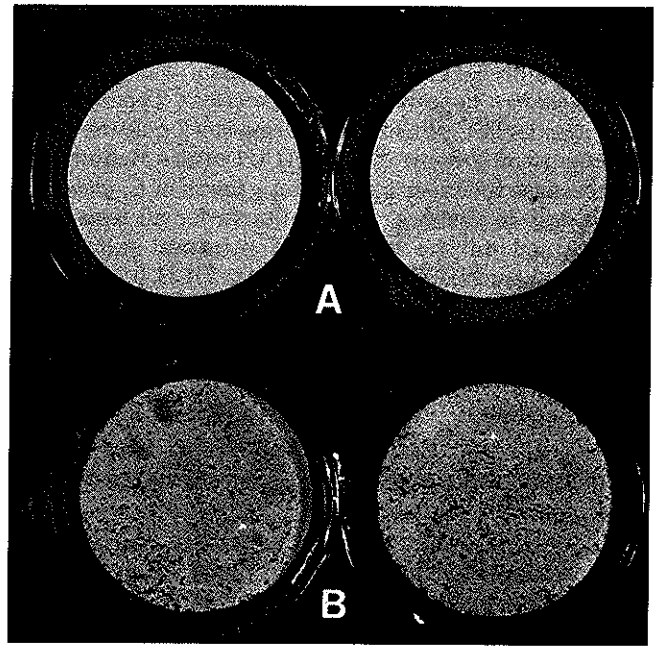
The "conditions of use" can include environmental conditions that favor fungal growth as well as contaminants that are capable of supporting fungal growth on the filter media. If this is determined to be the case, it may be necessary to incorporate fungicides in very high concentrations into the media. A preferable alternative would be to change the filters regularly.

Face-mask applications provide a practical example of how conditions of use can influence fungal growth in filter media. With prolonged use, the interior of such masks provides an optimum setting (high moisture and materials exhaled by the wearer) for fungal growth. Fungal growth on such masks can have medical implications for the wearer, ranging from mild allergic reactions to serious, systemic fungal infections (e.g., lung infections caused by the fungus *Aspergillus*, resulting in aspergillosis).

3. Control showing typical growth of *C. globosum* on cellulosic filter paper



4. Comparison of growth on filter media for (A) filtered inoculum and (B) unfiltered inoculum.



C. globosum is definitely not the fungus of choice for the application being considered here. Better choices might include other test fungi listed in Table 508.3-II of MIL-STD-810D, Method 508.3 (19 July 1983), or in Designation G21-70 of the American Society for Testing and Materials. There is also an array of other potential candidates, depending on the situation. Each of these two procedures provide for filtration of the inoculum similar to the method we described here. However, in these procedures, the filtration is carried out simply to remove mycelial fragments and clumps of agar. While MIL-F-51079D did not specify a filtration procedure for *C. globosum*, the need for such filtration is obvious, since cellulosic material can support growth of this fungus.

MIL-F-51079D was amended on February 17, 1988, to include the filtration procedure of MIL-STD-810D and ASTM G21-70. Although the reason given for the amendment was to "remove large mycelial fragments and clumps of agar," the filtration procedure will also remove the cellulose fiber that supported the growth of *C. globosum* and rendered the test results invalid.

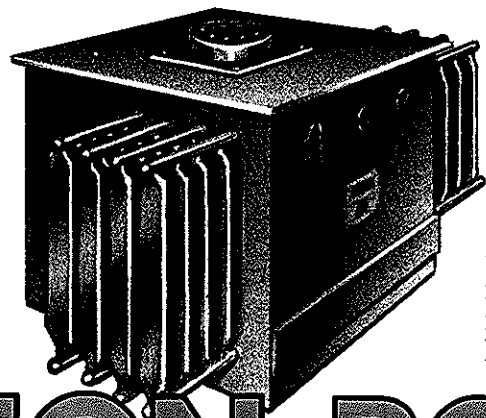
On a final note of caution: It may be a common practice to incorporate fairly high levels of fungicide into some filter media. It is likely that this practice was introduced to enable filter media to pass the mildew-resistance test as it was performed previously. To the best of our knowledge, no fungicides have been approved for such use. This is especially important if humans are to come in contact with any material filtered by such media. Since most fungicides are highly toxic to humans and may be carcinogenic or have other serious side effects, the potential health hazard from their misuse could be significant.□

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