Viability Control for Mildew Testing of Materials and Equipment in Tropical Chambers

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A method is proposed for indicating proper environmental conditions for fungus development in specification tests of materials in "tropical chambers."

It is common practice in the testing of materials for resistance to mold growth to expose them to warm humid conditions in test chambers (i.e., "tropical rooms") for extended periods of time. Testing specifications such as MIL-STD-810B (3) and others call for temperatures of approximately 30 C and relative humidities of approximately 95%. Development of mold on susceptible materials in the chamber may be delayed or prevented if these environmental conditions are not satisfied. In large chambers designed to accommodate military equipment, machinery, electronic components and the like, uniformity of conditions throughout the chamber is maintained with difficulty, and there may be local areas which are too dry or areas where moisture may be excessive. To monitor the prevailing conditions, it is customary to include so-called viability controls in the test; failure of these controls to become moldy within a specified time is evidence of suboptimal conditions.

Control materials have included such substrates as vegetable-tanned fungicide-free leather, protein-glue bonded cork, cloth impregnated with various nutrients, etc. None of these materials has been entirely satisfactory. This note describes a recently developed substrate which is reliable, easily prepared, and adaptable to a variety of tests. Strips of unsterilized cotton cloth are impregnated with a glycerol-mineral salts-yeast extract (GSY) solution. The cloth acts as a matrix-substrate complex, the glycerol as a humectant and nutrient, and the salts and yeast extract provide other necessary nutrients. In an atmosphere of 30 C and 95% relative humidity, visible mold colonies characteristically appear on this material in about 3 days, and in 5 to 7 days it is extensively molded. Unimpregnated cloth under similar conditions may require several weeks to develop light growth, and at relative humidities below 90% may remain mold-free for many weeks. The strips are placed adjacent to or within items, if possible, or long strips may be hung from the ceiling throughout the room, thus pinpointing local areas where molding conditions are suboptimal (Fig. 1).

The test solution (GSY), as finally developed, contained: 10% glycerol, 0.1% KH2PO4, 0.1% NH4NO3, 0.025% MgSO4·7H2O, and 0.05% yeast extract (pH 5.3). The fabric used was 8.25-oz (284.5 g/m²) bleached, scoured, cotton duck cut into strips [1.25-inch (3.2-cm) width]. The strips were dipped in the solution, excess liquid was expressed with the fingers, and the strips were then hung to air dry before being placed in the chamber.

The test chamber was about 20 ft wide by 25 ft long by 9 ft high. The temperature and humidity were maintained by conventional forced-draft equipment. The environmental conditions underwent a daily cycle with a 22-hr period at 30 C and 95% relative humidity and a 2-hr cool dry period when the temperature and relative humidity dropped to about 20 C and 75%, respectively.

The glycerol concentration was selected after testing a GSY series containing 0, 0.1, 0.5, 1, 2, 5, 10, and 50% glycerol, with salts and yeast extract concentrations as given above. Mold growth appeared most rapidly and was most dense on the strips which had been treated with 10% glycerol; this concentration was adopted as standard. Lesser growth appeared on the 1, 2, 5, and 50% strips and growth was practically absent from the 0, 0.1, and 0.5% strips even after 3 weeks of incubation.

To test the effect of the salts and yeast extract, a series of strips was prepared containing 0, 2, 4, 8, and 16% glycerol, with and without salts and yeast extract. After 7 days in the chamber, the strips with glycerol plus salts all showed mold growth, with maximal growth on the 8% strips (strip 1, Fig. 2). The strips without salts and yeast extract but with glycerol alone showed much less mold growth at 7 days (strip 2, Fig. 2)
and did not develop appreciable growth for several weeks. Strips treated with salts and yeast extract (no glycerol) or with distilled water showed no growth in 7 days (strips 3 and C, Fig. 2).

The adequacy of natural inoculum in the chamber was evaluated by comparing (i) strips treated with GSY without aseptic precautions and without inoculation, (ii) autoclaved strips treated with sterile GSY and handled aseptically until placed in the chamber, and (iii) strips treated as in procedure (i) but inoculated by spraying with a mixture of spores (2). At the end of 1 week, all strips showed extensive mold growth, indicating that the normal flora in the atmosphere of the chamber was adequate and that no beneficial effect was obtained by supplementary inoculation.

To test the response of the GSY strips to different humidities, a series was suspended in desiccators at 30 C over saturated salt solutions selected to give 100, 96.5, 91, and 84% relative humidity (1). Growth was abundant after 1 week in atmospheres of 91% or higher. Growth at 84% did not approach that in the higher humidities until after 3 weeks of incubation. This indicates the order of humidities required, although it is recognized that the static situation in a closed container may not be exactly comparable to experimental conditions.

A comparison of the GSY treatment with that specified in the IEC method (4) in which cotton duck is impregnated with 2% malt extract showed the latter to be essentially free of growth at 1 week (strip T4, Fig. 2), whereas the GSY strips were well molded. At 20 days, both GSY and malt extract strips showed extensive growth.

This method is suggested as a standard viability control for use in chamber testing of materials and equipment for susceptibility or resistance to mold growth.

**LITERATURE CITED**