GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 83.080.01

G 31

GB/T 24128-2018 / ISO 16869:2008

Replacing GB/T 24128-2009

Plastics – Assessment of the Effectiveness of Fungistatic Compounds in Plastics Formulations

(ISO 16869:2008, IDT)

塑料 塑料防霉剂的防霉效果评估

Issued on: December 28, 2018 **Implemented on: November 01, 2019**

Issued by: State Administration for Market Regulation;
Standardization Administration of the People's Republic of China.

Table of Contents

Plastics – Assessment of the Effectiveness of Fungistatic Compounds in Plastics Formulations

WARNING - Handling and operating potentially hazardous microorganisms requires high technical competence and must comply with existing national laws and regulations. These tests shall only be performed by personnel trained in microbiological techniques. Relevant disinfection, sterilization and personal hygiene practices shall be strictly implemented.

1 Scope

This Standard specifies a test method for determining the antifungal effectiveness of fungistat used in plastic formulations to protect susceptible ingredients such as plasticizers and stabilizers. This method can verify whether a certain plastic product can effectively prevent the erosion of fungi.

The antifungal effect is evaluated by visual inspection.

This Standard applies to films or sheets made of plastics up to 10mm thick. In addition, porous materials such as foamed plastics can also be tested by using this method when made into the above forms.

The fungistat must have certain dissolution properties in the matrix.

Different from ISO 846, this Standard does not spray fungi spore suspension on the surface of the sample, but covers a layer of spore-containing agar. This shall better disperse the spores and provide the moisture necessary for spore germination on plastic surface that is normally hydrophobic.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this document.

ISO 291:2008 Plastics – Standard Atmospheres for Condition and Testing

3 Terms and Definitions

For the purposes of this Document, the following terms and definitions apply.

3.1 Plastic susceptible to fungal attack

Plastic that contains in its formulation one or more nutrients that support fungal growth.

3.2 Fungistat

Compound that prevents fungal growth on a material that is normally susceptible to fungal attack.

4 Principle

Expose the surface of the specimen to the fungi spore suspension; and disperse the spores in a thin layer of agar medium without adding carbon source on the surface of the test sample, which can ensure uniform dispersion of the spores and appropriate water supply.

Plastics without adding fungistat shall lead to the germination and initial growth of fungi spores. When there are components that are easily attacked by fungi in the plastic and no effective fungistat is added in the formulation, fungi spores shall further grow on and around the surface of the specimen and produces spores.

Plastics containing fungistat shall inhibit spore germination and initial growth on and around the specimen surface. The fungistat can dissolve into the agar surrounding the specimen, resulting in a larger bacteriostatic ring that inhibits spore germination and growth.

Although the bacteriostatic ring is not used as the evaluation index of the test results, it can still show the antibacterial effect of the fungistat.

5 Instruments and Materials

5.1 Instruments

Sterilize all glassware and other instruments that come into contact with media and/or reagents (except sterile instruments) by one of the following methods:

Method A: use an autoclave (see 5.1.2), sterilize at 121°C for at least 15min;

Method B: use a dry heat sterilizer (see 5.1.2), sterilize at 180°C for at least 30min, sterilize at 170°C for at least 1h, or sterilize at 160°C for at least 2h;

Method C: use a membrane filtration system with a pore size of 0.45μm.

5.1.1 Incubator, the temperature can be maintained at 24℃±1℃, and the relative humidity can be maintained at 85% or above.

5.1.2 Sterilization equipment

5.1.2.1 Moist heat sterilization, suitable autoclave.

5.1.2.2 For dry heat sterilization, the thermal oven shall maintain a temperature specified in Method B.

5.1.2.3 Membrane filtration sterilization, a membrane filtration device with the pore size specified in Method C.

5.1.3 Analytical balance, accurate to ±0.1mg.

5.1.4 Laboratory centrifuge, with the speed of 2000r/min~5000r/min.

5.1.5 Counting plate (direct counting under microscope).

5.1.6 Microscope, the magnification can reach 100×.

5.1.7 pH meter, with an accuracy of ±0.1pH unit, with temperature calibration function.

5.1.8 Vortex shaker, with the speed of 2000r/min~2500r/min.

5.1.9 Glass containers: test tubes, flasks or bottles of appropriate capacity.

5.1.10 Culture dish, with a diameter of 90mm ~ 100mm and a depth of at least 15mm.

5.1.11 Standard pipettes, with standard volume of 1.0mL and 15.0mL, calibrated automatic pipettes.

5.1.12 Standard graduated cylinder, with a minimum capacity of 30mL.

5.1.13 Glass beads, with diameter of 3mm~5mm.

5.2 Medium and reagents

All reagents shall be analytically pure and/or suitable for use in microbiological assays.

5.2.1 Water

The used water shall be distilled or deionized water with a conductivity of less than 1μS/cm.

5.2.2 Malt extract agar (MEA)

Malt extract: 30.0g;

Soy peptone: 3.0g;

5.3.1.3 Paecilomyces varioti: CGMCC3.4253 or CBS628.66;

5.3.1.4 Penicillium funiculosum: CGMCC3.3875 or ATCC9644;

5.3.1.5 Trichoderma longibrachiatum: CGMCC3.4291 or ATCC13631.

Other test fungus (such as Aspergillus terreus, Aureobasidium pullulans) can be added according to product use and needs, and all used strains shall be listed in the test report.

NOTE: CGMCC is the China General Microbiological Culture Collection Center; ATCC is the American Type Culture Collection; and CBS is the Centraalbureauvoor Schimmeicultures.

5.3.2 Culture conditions

In 5.3.1.1 and 5.3.1.3 to 5.3.1.5, the strain shall be cultured on the slant of malt extract agar (5.2.2) in a test tube; and culture at 24℃ ± 1℃ for 14 days ~ 21 days.

In 5.3.1.2, the strain shall be cultured on Chaetomium globosum agar (5.2.3) medium; and culture at 24℃ ± 1℃ for 14 days ~ 21 days.

Cultures for storage shall be kept on agar slants, preferably lyophilized or cryopreserved.

6 Test Sample

6.1 Shape and size

Sterilize the slicer that meets the requirements; and cut out a circular piece with a diameter of 1cm ~ 4cm or a square piece with a side length of 1cm ~ 4cm from each test sample as required. The thickness of the specimen shall not exceed 10mm.

6.2 Number of specimens

At least 3 specimens are prepared for each test sample for test evaluation.

7 Preparation of Specimen

7.1 Cleaning

Hold the test sample with tweezers; wash it repeatedly (with a brush if necessary) and store it in a clean container and dry it naturally. Use tweezers for all subsequent processing to avoid sample contamination.

7.2 Labeling and storage

Labels or markers may interact with the plastic during testing; so store samples separately in airtight containers (such as culture dishes) at room temperature; label the container rather than

the sample.

8 Test Procedures

8.1 Test temperature

Prepare and adjust the state of the sample according to the Class-2 atmospheric conditions [temperature 23℃ ± 2℃ and relative humidity (50 ± 10) %] in ISO 291:2008.

8.2 Pour the medium

After sterilization, pour 20 mL of nutrient salt agar (5.2.7) into each culture dish; allow it to solidify and dry until there is no visible moisture on its surface.

8.3 Placement of specimen

Place the test specimen discs separately, as flat as possible, in the middle of the solidified medium.

If the thickness of the specimen exceeds 5mm, punch holes in the agar with a hole punch of the same size as the specimen; and put the specimen into the agar hole. The punch should be sterilized, such as flame sterilization.

8.4 Inoculation of specimen

8.4.1 Preparation of spore suspension

Prepare a mixed spore suspension from a cultured spore culture as follows:

Add (see 5.3.2) 5 mL of wetting agent (5.2.4) to each culture tube; gently scrape the surface of the spore medium with a sterile inoculation loop to obtain a spore suspension; and gently shake the culture tube to disperse the spores; pour the spore suspension into a sterile conical flask containing 10 ~ 20 glass beads; and repeat the washing for three times according to the above process. Each spore suspension with glass beads is then shaken with a shaker (5.1.8) and filtered through a thin layer of sterile cotton or glass wool to eliminate residual hyphae.

Centrifuge the filtered spore suspension aseptically and discard the supernatant. Resuspend the sediment with 50 mL of nutrient salt solution (5.2.6); centrifuge again; and suspend the sediment with 100 mL of the same solution (5.2.6).

Measure the concentration of each spore suspension with a counting plate, and the number of spores shall be at least 5×10^6 pieces/mL.

Before use, mix together equal amounts of each spore suspension and mix with a shaker (5.1.8). Single spore suspensions can be stored for up to 4 days at 4°C; up to 2 months at -18°C; or up to 12 months at -196°C.

This is an excerpt of the PDF (Some pages are marked off intentionally)

------ The End ------