Designation: G22 – 23

Standard Practice for Determining Resistance of Plastics to Bacteria[1]

This standard is issued under the fixed designation G22; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers two procedures, A and B, for determining the effect of bacteria on the properties of plastics in the form of molded and fabricated articles, tubes, rods, sheets, and film materials. Procedure B provides a more extensive contact between the test bacteria and the specimens than does Procedure A. Changes in optical, mechanical, and electrical properties may be determined by the applicable ASTM methods.

1.2 The values stated in SI units are to be regarded as the standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D618 Practice for Conditioning Plastics for Testing

G21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

3. Summary of Practice

3.1 The procedure described herein consists of the following steps:

3.1.1 Selection of suitable specimens for determination of pertinent properties,

3.1.2 Inoculation of specimens with suitable organisms,

3.1.3 Exposure of inoculated specimens under conditions favorable to growth,

3.1.4 Examination and rating for visual growth, and

3.1.5 Removal, sterilization, and evaluation of specimens.

NOTE 1—Since the procedure involves handling and working with bacteria that may be capable of infecting man, it is essential that personnel trained in microbiology perform the portion of the procedure involving handling of bacterial organisms and inoculated specimens.

4. Significance and Use

4.1 The resin portion of plastic materials is usually resistant to bacteria, in that it does not serve as a carbon source for the growth of bacteria. It is generally the other components, such as plasticizers, lubricants, stabilizers, and colorants that are responsible for bacterial attack on plastic materials. It is important to establish the resistance of plastics to microbial attack when plastics are used under conditions of high temperature and humidity favorable for such attack.

4.2 The effects to be expected are:

4.2.1 Surface attack, discoloration, and loss of transmission (optical).

4.2.2 Removal of susceptible plasticizers, modifiers, and lubricants, resulting in increased modulus (stiffness), changes in weight, dimensions, and other physical properties, and deterioration of electrical properties such as insulation resistance, dielectric constant, power factor, and dielectric strength.

4.3 Often the changes in electrical properties are due principally to surface growth and associated moisture, and to pH changes caused by products of bacterial metabolism. Other effects include preferential growths caused by nonuniform dispersion of plasticizers, lubricants, and other processing additives. Pronounced physical changes may be observed on products in film form or as coatings where the ratio of surface to volume is high, and where nutrient materials such as plasticizers and lubricants continue to diffuse to the surface as they are utilized by the organisms.

4.4 Since attack by organisms involves a large element of change due to local accelerations and inhibitions, the order of reproducibility may be rather low. To assure that estimates of

[1] This practice is under the jurisdiction of ASTM Committee G03 on Weathering and Durability and is the direct responsibility of Subcommittee G03.04 on Biological Deterioration.

Current edition approved Feb. 1, 2023. Published February 2023. Originally approved in 1976. Last previous edition approved in 1996 as G22 – 76 (1996) which was withdrawn January 2002 and reinstated in February 2023. DOI: 10.1520/G0022-23.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

behavior are not too optimistic, the greatest observed degree of deterioration should be reported.

4.5 Conditioning of specimens such as exposure to leaching, weathering, heat treatment, etc., may have significant effects on the resistance of plastics to bacteria. Determination of these effects is not covered in this document.

5. Apparatus

5.1 *Glassware*—Glass vessels are suitable for holding specimens when laid flat. Depending on the size of the specimens, the following are suggested:

5.1.1 For specimens up to 75 mm (3 in.) in diameter, 150 mm (6 in.) covered petri dishes.

5.1.2 For 75 mm (3 in.) and larger specimens, such as tensile and stiffness strips, large petri dishes, trays of borosilicate glass; or baking dishes covered with squares of window glass or other suitable covering.

5.2 *Incubator*—Incubating equipment for all test methods shall maintain a temperature of 35 °C to 37 °C (95 °F to 99 °F) and a relative humidity of not less than 85 %. Automatic recording of wet and dry bulb temperature is recommended.

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[3] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean distilled water.

6.3 *Nutrient-Salts Agar*[4]—This is a carbon-free culture medium used in Practice G21. Although intended for the cultivation of fungi, nutrient-salts agar will support growth of the test bacteria when carbon requirements are supplied by susceptible plastic materials. Prepare this medium by dissolving in 1 L of water the designated amounts of the following reagents:

Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7 g
Potassium monohydrogen orthophosphate (K_2HPO_4)	0.7 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.7 g
Ammonium nitrate (NH_4NO_3)	1.0 g
Sodium chloride (NaCl)	0.005 g
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	0.002 g
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.002 g
Manganese sulfate ($MnSO_4 \cdot H_2O$)	0.001 g
Agar	15.0 g
Distilled water	1000.0 mL

Sterilize the test medium by autoclaving at 121 °C (250 °F) for 20 min. Prepare sufficient medium for the required tests including uninoculated controls.

NOTE 2—Nutrient-salts agar readily supports growth of fungi which may be present on the test specimens. Fungal contamination can be controlled by either (*1*) the addition of 0.15 % cycloheximide[5] to nutrient-salts agar or (*2*) sterilization of the specimens by some suitable means such as exposure to ethylene oxide.

6.4 *Bacterial Cell Suspension:*

6.4.1 The following test organism shall be used, or a suitable bacterium as agreed upon among parties concerned: *Pseudomonas aeruginosa* ATCC 13388,[6] MYCO B1468.[7]

6.4.2 Cultures of the organism shall be maintained on slants of nutrient agar.[4] To prepare nutrient agar slants suspend 0.3 % beef extract, 0.5 % peptone, and 1.5 % agar in distilled water and heat until dissolved. Tube, plug, and autoclave for 15 min at 103 kPa (15 psi) steam pressure at 121 °C. The tubed and sterilized media shall be allowed to cool and gel in a slanted position to afford an appropriate surface on which the bacteria may be cultured.

6.4.3 The inoculum shall be prepared from not less than two successive transfers in nutrient broth.[4] To prepare nutrient broth dissolve 0.3 % beef extract and 0.5 % peptone in distilled water and dispense in suitable test tubes or flasks. Plug and autoclave at 103 kPa (15 psi) steam pressure at 121 °C. Transfer the bacteria with a flame-sterilized needle from the nutrient agar slant to nutrient broth. Incubate for 24 h. Transfer this broth culture to the sterile nutrient broth medium and culture as before. Centrifuge the broth culture. Decant the broth and resuspend the bacteria cells in sterile normal saline solution (0.8 % NaCl). Centrifuge, decant the saline solution, and resuspend the bacteria cells in fresh normal saline. Determine the bacterial cell concentration.

6.4.4 The concentration of the bacterial cells may be estimated turbidimetrically using a photoelectric colorimeter. The turbidimetric standard is obtained by concurrent plate counts and turbidimetric measurements of a serially diluted bacterial cell suspension. Reference to a calibration curve of cell count versus absorption will give a measure of concentration. The cell count need be done only once to establish the calibration curve.

6.5 *Viability Control*—Streak onto sterile nutrient agar poured into a sterile petri dish a loop-full of the bacterial suspension at the start and end of the bacterial inoculation procedure. Contamination of this inoculum must be avoided. Incubate the viability culture at 35 °C to 37 °C (95 °F to 99 °F) at a relative humidity not less than 85 % for 48 h to 72 h. The inoculum shall be reported as viable only if typical growth occurs in this culture with no contamination. Absence of growth or the presence of atypical growth requires repetition of the test.

7. Test Specimens

7.1 The simplest specimen may be a 50 mm by 50 mm (2 in. by 2 in.) piece, a 50 mm diameter piece, or a piece (rod, tubing) at least 75 mm (3 in.) long cut from the material to be tested. Completely fabricated parts or sections cut from fabricated

[3] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[4] Agar, nutrient-salts agar, and nutrient broth are obtainable from biological laboratory supply sources.

[5] Similar or equivalent to "Actidione," a product of the Upjohn Co.

[6] American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

[7] Mycological Services, Box 126, Amherst, MA 01002.