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AMERICAN SOCIETY FOR TESTING AND MATERIALS
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Standard Test Method for Evaluating the Bacteria Resistance of Water-Dilutable Metalworking Fluids[1]

This standard is issued under the fixed designation D 3946; 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 test method covers evaluating relative bioresistance of aqueous metalworking fluids by challenging them with a biological inoculum prepared from specific deteriorated metalworking fluid from the user's site.

1.2 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 70 Test Method for pH of Aqueous Solutions with the Glass Electrode[2]

E 686 Method for Evaluation of Antimicrobial Agents in Aqueous Metalworking Fluids[3]

3. Significance and Use

3.1 This test method is applicable for determining in the laboratory the ratings of fluids as compounded from fluid manufacturers and for determining the need for biocide addition prior to utilization of the fluids in working systems.

4. Apparatus

4.1 *Air Compressor*—A suitable air compressor used to deliver compressed air for standard laboratory functions should be available. For purposes of this test the supply of air should be continuous and sufficient to aerate 1 L of fluid without excessive foaming.

4.2 *Incubator*—Ideally a cabinet used for maintaining temperatures at 30 ± 1°C should be used, but in the absence of such an instrument it is acceptable to incubate at ambient room temperature or at the same temperature at which the test system is run.

4.3 *Glassware*:

4.3.1 *French Square Bottles*, [4] 32 oz (960 mL), with cap. One bottle is needed for each test. This style of bottle is useful because it has a maximum height-to-surface area ratio and will maximally encourage anaerobic conditions during the performance of the test.

4.3.2 *Dilution Bottles*—Containers are needed for making appropriate dilutions when performing bacterial plate counts. Inexpensive, easily obtainable and sufficiently accurate are clear, graduated 6-oz (180-mL) screw-cap prescription ovals. Any other container sterilizable and with a holding capacity of approximately 200 mL is sufficient for making serial dilutions.[5]

4.3.3 *Pipets*, for making bacterial plate counts should have a rapid delivery to prevent settling in the pipet. The recommended pipets for this purpose are 2.2-mL bacteriological or dairy pipets. These are available presterilized and disposable.[6]

4.3.4 *Petri Dishes*, 100 by 15-mm, are used for making standard plate counts. These are available presterilized and disposable from a variety of sources.[7]

4.4 *Colony Counter*—Any one of several types may be used, although a colony counter is only a black-lighted surface with approximately 2× magnification for counting bacterial colonies and is not essential for the operation of this test.[8]

4.5 *Materials*:

4.5.1 *Bacteriological Medium*—Soybean casein digest agar USP is used for performing the bacterial plate counts.[9]

4.5.2 *Soybean Casein Digest Broth*—This medium is used to prepare the inoculum for challenging the fluids under test.[10]

4.5.3 *Biostix™*, can be used in Alternative Procedure B in

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[1] This test method is under the jurisdiction of ASTM Committee D-2 on Petroleum Products and Lubricantsand is the direct responsibility of Subcommittee D02.L on Industrial Lubricants.

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[2] *Annual Book of ASTM Standards*, Vol 15.05.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

[4] Thirty-two-ounce (960-mL) French square bottles are obtainable from VWR Scientific under their Catalog No. 16188-140.

[5] Clear, graduated 6-oz screw-cap prescription ovals are available from any pharmaceutical supply house. Standard milk dilution bottles (Corning Catalog No. 1372) are acceptable. Science Products Div., Corning Glass Works, Corning, NY 14830.

[6] Presterilized and disposable pipets are available from Corning, under their Catalog No. 5173.

[7] Petri dishes, available from a variety of sources, are obtainable from most local laboratory suppliers.

[8] The American Optical Counter is available from a large number of scientific equipment distributors, including Fisher, Scientific Products, and VWR.

[9] Soybean casein digest agar USP is available commercially as tryptic soy agar (DIFCO Corp); as trypticase soy agar (BBL). The procedure for preparation of the agar is listed in detail on the container in which the agar is sold.

[10] Tryptic soy broth (DIFCO Corp); trypticase soy broth (BBL).

place of the plate count procedure if this is desirable.[11]

4.5.4 *Metal Chips*—Although for most test methods metal chips will be ferrous,[12] other metals can be substituted if desirable in evaluating any particular operation.

4.6 *Sterilizer*—Any suitable sterilizer is acceptable, including pressure cookers with pressure indicators to ensure that appropriate conditions are maintained for sterilization.[13] This sterilizer is necessary for water dilution blanks and for the microbiological media.

5. Test Materials

5.1 Test materials will include the metalworking fluids for evaluation. Run at recommended dilutions or at contemplated dilutions.

6. Inoculum

6.1 *Preparation*—Deteriorated metalworking fluid from a location of interest is mixed with equal parts of sterile soybean casein digest broth to make a sufficient volume to perform all the subsequent evaluations. Prepare this inoculum fresh for each series of tests. The fluid broth mixture is incubated at ambient temperatures with aeration for 48 h.

6.2 *Evaluation*—The bacterial level in the inoculum must be checked before use and the count should not be less than 5×10^8/mL. This can be done either by the plate count method or by the alternative Biostix™ method, in which case that level can be determined by appropriate dilution of the test sample.

7. Procedure

7.1 *Preparation of the System*—The vessel used for running this test is a 1-qt (0.9-L) French square bottle. When filled to the brim it holds 1 L. Therefore, when initially setting up the experiment, in order to maintain the ratio of 1 part of inoculum to 10 parts of the test system, add test fluid after the addition of 10 g of metal chips and 100 mL of inoculum. Add fluid to be tested to the brim. This is a total volume of 1000 mL, of which 100, or 10 %, is the inoculum. Put the screw-cap cover in place and invert the container a sufficient number of times to allow for complete mixing. Then remove approximately 50 mL so that the level of liquid is just above the shoulder of the bottle.

7.2 *Aeration*—A capillary tube or one of the 2.2-mL pipets will suffice as an aerator. Connect to the compressed air source with the tube as close to the bottom of the uncapped fluid container as possible. Continue aeration for 5 days at which time make up any losses due to evaporation with distilled water or water of equal purity. Suspend aeration for the equivalent of a weekend shut-down, or 2½ days, and then resume for 5 subsequent days. Add water as before to make up for evaporation.

7.3 *Sample Regimen*:

7.3.1 Remove samples for bacteriological evaluation and for pH measurement four times during the course of this test: (*1*) prior to the initiation of aeration, (*2*) after the first 5 days of aeration, (*3*) after the first weekend shut-down, and (*4*) five days thereafter.

7.3.2 Any standard pH meter is suitable for measuring pH. Make pH measurements on the sample allowing for sufficient equilibration time when emulsion systems are being tested. Carefully clean electrodes between samples, especially when oil-containing samples are involved. See Section 3.

8. Bacteriological Evaluation

8.1 *Plate Count Method*—The standard plate count is the accepted method in many sanctioned areas for determining viable numbers of bacteria per millilitre. This involves making the necessary serial dilutions in 99 mL of sterile water with 1 mL of initial fluid. This primary dilution is then used for making subsequent dilutions (see Fig. 1). The procedure is adequately described in *Standard Methods for the Evaluation of Milk and Dairy Products* (13th edition). Since counts less than 10 000/mL are of minor significance in metalworking fluids, it is recommended that undiluted working fluid, 1 to 10, 1 to 100, and 1 to 1000, be omitted in preparing plate counts and that the first plates examined be at the level of 1 to 10 000. For purposes of comparison, the test can be run from 1 to 10 000 through 1 to 1 000 000. According to *Standard Methods*, the plates containing more than 30 or less than 300 colonies are used for determining the viable bacterial levels in the derived fluid. The colony count from such plates are multiplied by the inverse of the dilution factor to get the actual count. If there are more than 300 colonies on the 1 to 1 000 000 dilution plates, the results are given as more than 300. If the colony counts on the 1 to 10 000 plates are less than 30, the actual count is used times the dilution factor. If there are no colonies on the lowest dilution, that is 1 to 10 000, then the report reads less than 10 000/mL.

8.2 *Biostix™ Method*—This method can be used in place of standard plate count and involves only the immersion of a prepared media stick into a 1 to 100 dilution of test fluid. All of the instructions and materials are available with each kit of 25 Biostix™ test strips.

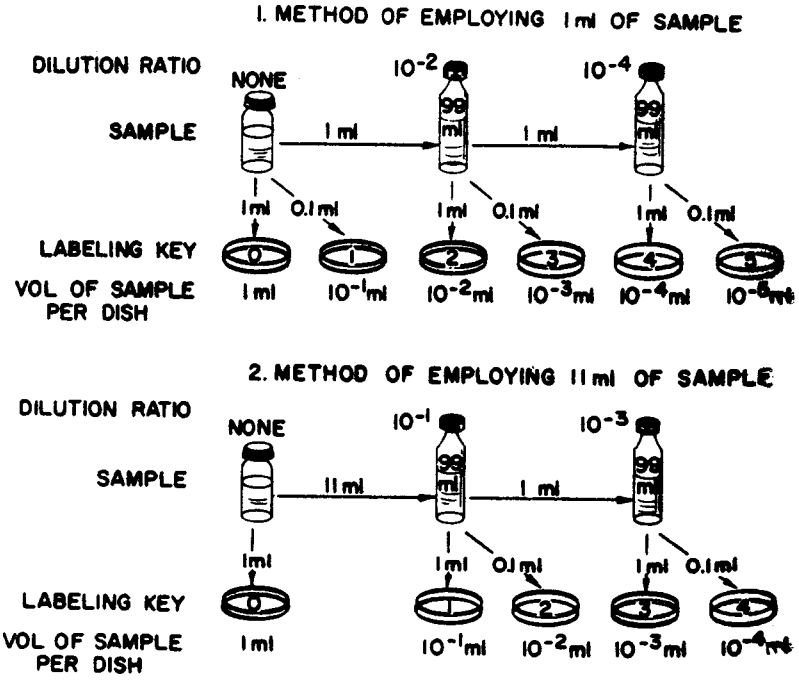
9. Evaluation of Results

9.1 This test is designed to give relative values rather than absolute values for fluid bioresistance. Different fluids may have different stabilities to large numbers of bacteria and, therefore, there is a greater variation in bacterial levels that cause physical and chemical changes in fluids. Thus, total count is only one parameter in monitoring biological acceptability. The drop in pH (see Test Method E 70) along with a rise in bacterial count, or a rise in bacterial count between readings, should be a warning that the fluid is starting to deteriorate biologically. The test is not only designed to evaluate shelf products as received from fluid manufacturers, but also as a rapid laboratory method for determining the best level of a suitable biocide that can be added to supplement the test formulation.

[11] Biostix are supplied by Biosan Laboratories, Inc., 10657 Galaxie, Ferndale, MI 48220.

[12] Ferrous metal chips are obtainable from Fisher, under their Catalog No. 20 mesh.

[13] Sears Roebuck or similar pressure cookers containing a pressure indicator are usually of sufficient size. These pressure cookers have a capacity of about 20 qt (19 L).



Preparation of dilutions.

NOTE 1—Reference Standard Methods for the Evaluation of Milk and Dairy Products (13th Edition), p. 78.

FIG. 1 Preparation of Dilutions

10. Precision and Bias [14]

10.1 *Precision*—The statistical distribution in microbiological counts usually do not follow normal distribution patterns (Reference Steiner, *Statistical Manual of the Association of Official Analytical Chemists*; Youdon, W. S., and Steiner, E. H., 1975, p. 82.).

10.2 *Bias*—The procedure in this test method for measuring bacteria resistance has no bias because the value of bacteria resistance is defined only in terms of this test method.

11. Pertinent References for Protocol

11.1 A related protocol is Method E 686, under the jurisdiction of Committee E-35.

11.2 The confirmation of the irrelevance of type of inoculum in establishing ratings has been published. (Refs: Rossmoore, H. W., Scezny, P., and Rossmoore, L. A., "Evaluation of Source of Bacterial Inoculum in Development of a Cutting Fluid Test Procedure," *Lubr. Engg*. 33(7): 372–377, July 1977; Rossmoore, H. W. and Rossmoore, L. A., "The Identification of a Defined Microbial Inoculum for the Evaluation of Biocides in Water-Based Metalworking Fluids," IN PRESS, *Lubr. Engg*., January 1980.)

12. Keywords

12.1 bacteria resistance; bacteria resistance test; metalworking; water-dilutable fluids

[14] The results of the cooperative round-robin test program are filed at ASTM Headquarters. Supporting data are available from ASTM Headquarters. Request RR:D02-1134.