



## Standard Test Method for Evaluating the Bacteria Resistance of Water-Dilutable Metalworking Fluids<sup>1</sup>

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<sup>2</sup>

E 686 Method for Evaluation of Antimicrobial Agents in Aqueous Metalworking Fluids<sup>3</sup>

### 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  $30 \pm 1^\circ\text{C}$  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*, <sup>4</sup> 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.<sup>5</sup>

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.<sup>6</sup>

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.<sup>7</sup>

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.<sup>8</sup>

#### 4.5 Materials:

4.5.1 *Bacteriological Medium*—Soybean casein digest agar USP is used for performing the bacterial plate counts.<sup>9</sup>

4.5.2 *Soybean Casein Digest Broth*—This medium is used to prepare the inoculum for challenging the fluids under test.<sup>10</sup>

4.5.3 *BioStix*<sup>TM</sup>, can be used in Alternative Procedure B in

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

<sup>5</sup> 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.

<sup>6</sup> Presterilized and disposable pipets are available from Corning, under their Catalog No. 5173.

<sup>7</sup> Petri dishes, available from a variety of sources, are obtainable from most local laboratory suppliers.

<sup>8</sup> The American Optical Counter is available from a large number of scientific equipment distributors, including Fisher, Scientific Products, and VWR.

<sup>9</sup> 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.

<sup>10</sup> Tryptic soy broth (DIFCO Corp); trypticase soy broth (BBL).

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<sup>2</sup> *Annual Book of ASTM Standards*, Vol 15.05.

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 11.05.