



Designation: E 686 – 91 (Reapproved 1998)

Standard Method for Evaluation of Antimicrobial Agents in Aqueous Metal Working Fluids¹

This standard is issued under the fixed designation E 686; 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 method evaluates the utility and effectiveness of antibacterial and antifungal agents at use concentrations in controlling the microorganisms responsible for the deterioration of metal-working fluids. The method is applicable to antimicrobial agents that are incorporated into either the metal-working fluid concentrate or into the final-use dilution.

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:

D 2881 Classification of Metal Working Fluids and Related Materials²

D 3946 Test Method for Evaluating the Bacteria Resistance of Water-Dilutable Metalworking Fluids²

3. Summary of Method

3.1 Antimicrobial agents are incorporated into a metal-working fluid at its use concentration. The fluid is inoculated with a microbial culture. The mixture is aerated for 5 days and then left unaerated for 2 days to simulate plant operations and to encourage anaerobiosis. The sample is microbiologically evaluated. The regimen of inoculating, aerating, and evaluating is continued for a minimum of 6 weeks. A knowledge of standard microbiological techniques is required for this procedure.

4. Significance and Use

4.1 This method should be used to determine the usefulness and the effective level of a microbicide in a metal-working fluid system.

5. Apparatus

5.1 *Air Supply* (Note 1).

5.2 *Colony Counter*.³

5.3 *Incubators*, 25°C and 35°C.

5.4 *Autoclave*.

5.5 *Glass and Plastic Ware* (Note 2):

5.5.1 French square bottles, 1-L.

5.5.2 Petri dishes, 100 by 15 mm.

5.5.3 Bacteriological pipets.

5.5.4 Counting bottles.

5.6 *pH Meter*.

NOTE 1—Any air source that is free of organic vapors, organic matter, or other objectionable material may be used. Sterile air need not be used for the undefined inoculum. If necessary, air may be sterilized as follows:

Pack two 150-mm long drying tubes (bulb-type) loosely with borosilicate glass wool in series with neoprene stoppers, glass tubing, and neoprene tubing. Wrap loosely in aluminum foil and steam sterilize at 15 to 20 psi (103 to 138 kPa) for 30 min or heat sterilize at 160°C for 2 h. Cool to room temperature while wrapped.

Insert into air line with bulbs on upstream side. Average lifetime in continuous use is 2 weeks. Discard sooner if upstream filter becomes wet or contaminated with oil.

NOTE 2—Sterile laboratoryware or sterile disposable laboratoryware should be used according to standard microbiological practice.

6. Materials

6.1 Metal Chips.

NOTE 3—Cast iron filings are suggested. If the metal working fluid is to be used in operations where other metals are used, it is recommended chips from these metals also be substituted.⁴ Chips should be prewashed with toluene (or similar non-polar solvent), then methanol (or similar polar solvent) and dried before use.

6.2 Antimicrobial Agent(s).

NOTE 4—Since efficacy in a medium as complex as a metal-working fluid is more a relative than absolute measurement, it is prudent to always evaluate at least two antimicrobial treatments. Preferably one treatment should serve as a positive control; its efficacy in the test system having

¹ This method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents. Current edition approved Aug. 15, 1991. Published October 1991. Originally published as E 686 –80. Last previous edition E 686 –85.

² *Annual Book of ASTM Standards*, Vol 05.02.

³ The Quebec colony counter, or equivalent, has been found suitable for this purpose.

⁴ Iron filings, 40-mesh, may be obtained from either Curtin Scientific Co., Catalog No. 1083-080407 or Fisher Scientific Co., Catalog No. I-57.



been established previously.

6.3 Metal-Working Fluid(s).

NOTE 5—The number of metal-working fluids available is almost limitless. Recommendations for the use of any particular fluid cannot be made, but it is suggested the biocide be evaluated in various types of formulations. Refer to ASTM Committee D-2 on Petroleum Products and Lubricants for list of types of fluids. Classification D 2881 and Test Method D 3946 also provide information concerning fluids.

6.4 *Water*, 100 to 150 ppm hardness as calcium carbonate for the dilution of the metal-working concentrate(s).⁵

6.5 *Microbiological Media*—General retrieval media consistent with good microbiological practices are acceptable. Examples are:

6.5.1 Soybean-casein digest agar (USP XXI, Medium II).

6.5.2 Fluid soybean-casein digest (USP XXI, Medium III).

6.5.3 Sabouraud dextrose agar (USP XXI, Medium 19).

6.5.4 Sabouraud dextrose broth (USP XXI, Medium 20).

6.5.5 American Petroleum Institute (API) anaerobic media (sulfate API broth [BBL, Difco or other reputable biological growth media supplier]). Supplement the formulation with 0.1 g/L of ferrous ammonium sulfate, 0.6 g/L of sodium sulfite and 3.0 g/L of agar. Dispense to top of 1-oz screw-capped bottles. Sterilize.

6.6 *Inoculum*—The microbial inoculum may vary according to the user's requirements. It may be either undefined or defined. The challenge population should be acclimated to the coolant before being used in this method. Acclimatization is achieved by growing the challenge microbes in biocide-free, working-dilution coolant.

6.6.1 Prepare an undefined inoculum by adding 100 mL of spoiled cutting fluid to 900 mL of freshly prepared, biocide-free cutting fluid. Aerate at 25°C for 24 h or until the microbial count reaches 10⁹ CFU/mL. Replace 900 mL of this cutting fluid with freshly prepared, biocide-free cutting fluid. Aerate at 25°C for 24 h or until the microbial count reaches 10⁹ CFU/mL. Replace 900 mL of this cutting fluid with freshly prepared cutting fluid. Repeat the aeration and coolant replacement procedure for a minimum of three cycles before using the preparation as an inoculum.

6.6.2 Prepare a defined inoculum by using standard microbiological techniques to isolate specific cultures from spoiled metal-working fluid, then cultivate them in biocide-free cutting fluid, following the same procedure outlined for undefined inocula (see 6.6.1). Other microorganisms of particular interest may be used similarly. Examples include: *Staphylococcus aureus* (ATCC 6538), *Desulfovibrio desulfuricans* (ATCC 7757), *Pseudomonas aeruginosa* (pyocyanin positive), *Pseudomonas oleovorans* (ATCC 8062), *Klebsiella pneumoniae* (ATCC 13883), *Escherichia coli* (ATCC 8739), *Proteus mirabilis* (ATCC 4675), and *Fusarium sp.* (Rossmoore strain).

NOTE 6—As more “bioresistant” metal-working fluid formulations are developed, biocide-free cutting fluid may not support microbial growth. If microbial counts do not increase by at least three logs within 48 h (for example, 10⁴ CFU/mL at time 0; >10⁷ CFU/mL at time 48), then the

coolant should be augmented with 1 part in 10 of fluid soybean-casein digest.

7. Procedure for Evaluating Antimicrobials to be Applied in-Drum

7.1 Add appropriate concentrations of test biocide(s) to aliquots of coolant concentrate.

7.2 Dilute sufficient coolant concentrate from each treatment (including untreated control) to set up test system. Typically 1 to 2 L of each is adequate.

7.3 To 1-L French square bottles (900-mL working capacity), add 9 g of metal chips and 900 mL of metal-working fluid at its use concentration.

NOTE 7—If tramp oil or other organic material is known to be a contaminant in a metal-working fluid system, such material may be added to the test metal-working fluid.

7.4 Add 100 mL of inoculum and mix.

7.5 Remove and discard 100 mL of the total quantity.

7.6 Allow to sit undisturbed for 64 h. Mix and sample for microbiological testing.

7.6.1 Enumerate the bacteria or fungi, or both, by standard plate-counting techniques using soybean-casein digest agar or Sabouraud dextrose agar, or both.

7.6.2 Examine for sulfate reducers by inoculating 1 mL of the metal-working fluid into the bottom of the API media. Cap tightly and incubate for 2 weeks at 25°C. Observe periodically for blackening of the media which indicates the presence of anaerobic sulfate reducers.

7.7 Aerate the mixture using capillary tubing to bubble air into the bottom of the bottle (Note 8). If necessary, an antifoam may be added.⁶

NOTE 8—Air may be introduced by means of 2.2-mL cotton-plugged pipets or equivalent.

7.8 After 5 days, discontinue the aeration, replace all losses due to evaporation with sterile deionized or distilled water, and mix.

7.9 Allow to sit for 64 h and mix.

7.10 Remove 90 mL of the fluid and evaluate for microbial content in accordance with 7.6.1 and 7.6.2.

7.11 Reinoculate with 10 mL of the inoculum described in 6.6.

7.12 Replace all losses due to fluid removal with fresh metal-working fluid containing sufficient biocide to maintain the original concentration.

7.13 Determine the pH of the fluid at the start of the test and every 7 days thereafter.

7.14 Observe the physical condition of the fluid at the start of the test and every 7 days thereafter.

7.15 Resume aeration and repeat the regimen for a minimum of 6 weeks or until failure of the biocide occurs.

⁵ A formula for the preparation of hard water may be found in *Methods of Analysis*, AOAC, Fourteenth Ed., 1984, pp. 70–71.

⁶ The sole source of supply of the apparatus known to the committee at this time is Foam-trol 103, available from Arlen Chemical Corp., 198 Pacific St., Newark, NJ 07114. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend.

