Designation: E 2250 – 02

An American National Standard

Standard Method for Determination of Endotoxin Concentration in Water Miscible Metal Working Fluids[1]

This standard is issued under the fixed designation E 2250; 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 covers quantitative methods for the sampling and determination of Gram-negative bacterial endotoxin concentrations in water miscible metalworking fluids (MWF).

1.2 Users of this method should be familiar with the handling of MWF.

1.3 This method gives an estimate of the endotoxin concentration of the sampled MWF.

1.3.1 Used on site, this method gives an indication of changes in Gram-negative bacterial contamination in the MWF.

1.3.2 This method does not replace Practice E 2144.

1.4 This method seeks to minimize inter-laboratory variation but does not ensure uniformity of results.

1.5 This method is not intended to relate endotoxin concentration in MWF to health effects of inhaled endotoxin.

1.6 *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 1356 Terminology Relating to Sampling and Analysis of Atmospheres[2]

D 4840 Guide for Sample Chain-of-Custody Procedures[3]

E 1497 Practice for Safe Use of Water-Miscible Metal Removal Fluids[2]

E 1542 Terminology Relating to Occupational Health and Safety[2]

E 2144 Practice for Personal Sampling and Analysis of Endotoxin in Metalworking Fluid Aerosols in Workplace Atmospheres[2]

2.2 *Government Standard:*

29 CFR 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories[4]

2.3 *Other Documents:*

Criteria Document for a Recommended Standard: Occupational Exposure to Metalworking Fluids, 1998

NIOSH Manual of Analytical Methods (NMAM), 4th ed., Eller and Cassinelli, Eds., 1994[5]

3. Terminology

3.1 For definitions of terms in this method relating to the subset of metalworking fluids that are used for machining or grinding to produce the finished part, refer to Terminologies D 1356 and E 1542.

3.2 For definitions pertaining to endotoxins refer to Practice E 2144.

3.3 Definitions of Terms Specific to This Standard:

3.3.1 *Endotoxin neutralizing protein (ENP), n*—a recombinant DNA-produced protein that is labeled with a fluorophore and is capable of binding endotoxin.

3.3.2 *Fluorescence Polarization (FP), n*—an optical technology that relates planar fluorescence to molecular volume and specific analyte concentration.

3.3.2.1 *Discussion*—In this context, the molecular volume of ENP is related to endotoxin concentration.

3.3.3 *Tracer*—The term used to indicate fluorophore-labeled ENP.

3.3.4 *mP, n*—The mathematical expression of the planar fluorescence used in fluorescence polarization.

3.3.5 *endotoxins, n*—a lipopolysaccharide derived from the outer membrane of Gram-negative bacteria.

3.3.6 *endotoxin unit (EU), n*—a biological potency unit equivalent to the FDA Reference Standard Endotoxin (RSE). Currently, EC-6 is equivalent to 0.1 ng per 1 EU.

3.3.7 *Limulus amebocyte lysate (LAL) assay, n*—a biological assay that detects endotoxin.

3.3.8 *Gram-negative bacteria, n*—prokaryotic cells that have a complex cell-wall structure that stain characteristically when subjected to the differential Gram staining procedure.

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[1] This method is under the jurisdiction of ASTM Committee E34 on Occupational Health and Safety and is the direct responsibility of Subcommittee E34.50 on Health and Safety Standards for Metal Working Fluids.

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[2] *Annual Book of ASTM Standards*, Vol **11.03**.

[3] *Annual Book of ASTM Standards*, Vol **11.01**.

[4] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.

[5] CDC/NIOSH, 4676 Columbia Pkwy, Cincinnati, OH 45226–1998

4. Summary of Method

4.1 A known volume of MWF, typically 1 to 10 µL, is dispensed into a 10 × 75 mm borosilicate glass test tube containing 1-2 mL of endotoxin-free water.

4.2 The diluted MWF, without added tracer, is used to determine the base fluorescence polarization intensity.

4.3 The fluorescent-labeled endotoxin neutralizing protein (tracer) is added to the sample to determine the net planar fluorescence intensity, which is directly related to the endotoxin concentration.

5. Significance and Use

5.1 Practice E 2144 uses a biological test (LAL) to measure air-borne endotoxin. The LAL test is not only time consuming and difficult to perform but is also subject to interferences, for example, biocides, which may render it unreliable to measure MWF endotoxin.

5.2 Air-borne endotoxin found in metal processing operations is directly related to the endotoxin concentration in the MWF. A simple, rapid on site method to monitor MWF endotoxin will assist users attempting to incorporate measures to control or limit air-borne endotoxin.

5.3 A standard method for measuring MWF endotoxin concentrations will help to foster a better understanding of endotoxin exposure-response relationships.

6. Interferences

6.1 ENP is a positively charged protein with a portion of it (termed the "Loop") capable of binding the endotoxin molecule. In some cases, other negatively charged molecules are attracted to the Loop causing an increase in fluorescence polarization and resulting in falsely high levels of endotoxin in the MWF. One such group of molecules found in MWF is sulfonates. Sulfonate interference is determined by testing virgin MWF diluted with endotoxin-free water. If positive, a special sulfonate binding peptide is used to minimize the interference in the assay.

6.2 Certain dyes may interfere with fluorescence intensity measurements. The most commonly used dye causing interference in the green area of the spectrum is fluorescein.

6.3 Sulfonates are often used as emulsifiers, or surfactants, or both in MWF.

7. Apparatus

7.1 *Sampling*:

7.1.1 *Micropipette*, a pipette designed to dispense 1 to 10 µL of MWF.

7.2 *Vortex Mixer*, general purpose with a minimum speed of 500 rpm.

7.3 *Measurement*, a fluorescent polarization instrument with the capability to measure fluorescein (480-490 nm excitation - 505-520 nm emission wavelength).

8. Reagents and Materials

8.1 *Control Standard Endotoxin (CSE)*—Endotoxin preparations used for calibration standards shall be referenced to the FDA Reference Standard Endotoxin (RSE), which is presently EC-6 RSE. Calibration standards data and corresponding regression data are expressed in EU.

8.2 Endotoxin detection reagents, utilized in accordance with manufacturer's instructions.

9. Hazards

9.1 Aerosols of endotoxin preparations pose a potential respiratory hazard to susceptible laboratory personnel who are involved with an endotoxin assay directly.

9.2 Follow good laboratory procedures for worker protection and waste disposal, including 29 CFR 1910.1450.

9.3 Inhalation or dermal exposure to MWF may pose health problems for personnel involved in aerosol sampling. Provision of personal protective equipment (PPE) in the form of respirators or protective clothing, or both, may be indicated (see Practice E 1497 and Criteria Document for a Recommended Standard: Occupational Exposure to Metalworking Fluids).

9.4 Review material safety data sheets (MSDS) for materials in use at a facility to identify potential hazards to determine appropriate PPE (see 29 CFR 1910.1000).

10. Sampling

10.1 Obtain a sample of MWF by collecting a representative sample from an outlet valve into a clean and previously unused bottle. At least a 10–mL aliquot should be obtained from which the 1 to 10–µL sample for endotoxin measurement is obtained.

11. Storage and Shipment

11.1 Store the used MWF at 4 ± 2°C until shipped or analyzed. Do not freeze the sample at any time.

11.2 Samples should be shipped via overnight delivery. If the shipment will take more than 24 h to arrive at its destination, ship the samples in a Styrofoam container with cold packs.

11.3 Maintain procedures for sample custody in accordance with accepted chain of custody procedures (see Guide D 4840).

11.4 Upon receiving the sample(s), laboratory personnel shall record the date and time of receipt of the sample(s).

12. Extraction

12.1 Allow the sample to reach ambient temperature (approximately 20°-25°C/68°-77°F).

12.2 Vigorously shake or mix by vortexing the sample bottle before removing an aliquot for endotoxin analysis.

13. Calibration and Standardization

13.1 A fluorescence polarization instrument should be used in accordance with the manufacturer's instructions.

13.2 Maintenance and repairs of the sampling and analytical equipment should be performed in accordance with the recommendations of the manufacturer and should be documented in maintenance records.

13.3 Polarization standards supplied with an FP instrument should be used to insure instrument is performing properly.

13.4 Fluorescent endotoxin detection reagents for use with an FP instrument should be tested in accordance with the manufacturer's package insert.

14. Procedure

14.1 Analysis of the MWF sample using FP endotoxin detection reagents shall be performed in accordance with the manufacturer's instructions.

15. Quality Assurance

15.1 Ensure validation and maintenance procedures have been conducted in accordance with the fluorescence polarization instrument's manufacturer's instructions.

15.2 The correctness of software calculations shall be validated at least annually by checking selected generated data with other software or calculators.

15.3 Individuals who perform endotoxin assays shall be appropriately trained. Good laboratory quality assurance procedures should be in place.

15.4 Ensure that polarization standards and ENP tracer and other quality control measures meet manufacturer's specifications.

16. Calculation or Interpretation of Results

16.1 Endotoxin concentration of standards and samples shall be determined in accordance with the kit manufacturer's instructions.

16.2 Resultant endotoxin concentration data shall be expressed in endotoxin units per mL (EU/mL) of MWF.

16.3 Currently there are no data supporting a chemically, or clinically relevant, or both endotoxin level for MWF.

17. Report

17.1 Report the following information as a minimum:

17.1.1 The type of MWF.

17.1.2 The location and date the sample was taken.

17.1.3 The time since the last biocide addition.

17.1.4 The biocide, if any, that was added.

17.1.5 The concentration of endotoxin in the sample in EU per mL.

17.1.6 The base mP of the virgin MWF diluted to the appropriate working concentration with endotoxin-free water. This base mP must be determined only once for any given MWF and referenced thereafter.

17.1.7 Any variance from the stipulated procedure in conducting the test. This could include but is not limited to items such as:

17.1.7.1 Inability to obtain a readable result for any reason including, but not limited to color and presence of sulfonates in the MWF.

18. Precision and Bias

18.1 *Precision*—The precision and reproducibility of this test method are being determined and will be available on or before January, 2007. It is not feasible to specify the precision of the procedure at this time because data comparing various types of MWF are not yet available. However, reproducibility of this test method when used to detect endotoxin in water is ±10 %.

18.2 *Bias*—No information can be presented on the bias of the method until precision and reproducibility information are developed for MWF.

19. Keywords

19.1 endotoxin; endotoxin assay; endotoxin units (EU); fluorescence polarization; Gram-negative bacteria; MWF