



Standard Method for Determination of Endotoxin Concentration in Water Miscible Metal Working Fluids¹

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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 Atmospheres²

D 4840 Guide for Sample Chain-of-Custody Procedures³

E 1497 Practice for Safe Use of Water-Miscible Metal Removal Fluids²

E 1542 Terminology Relating to Occupational Health and Safety²

E 2144 Practice for Personal Sampling and Analysis of Endotoxin in Metalworking Fluid Aerosols in Workplace Atmospheres²

2.2 Government Standard:

29 CFR 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories⁴

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., 1994⁵

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 amoebocyte 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.

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