



Standard Practice for Personal Sampling and Analysis of Endotoxin in Metalworking Fluid Aerosols in Workplace Atmospheres¹

This standard is issued under the fixed designation E 2144; 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 practice covers quantitative methods for the personal sampling and determination of bacterial endotoxin concentrations in poly-disperse metalworking fluid aerosols in workplace atmospheres. Users should have fundamental knowledge of microbiological techniques and endotoxin testing.

1.2 Users of this practice may obtain personal or area exposure data of endotoxin in metalworking fluid aerosols, either on a short-term or full-shift basis in workplace atmospheres.

1.3 This practice gives an estimate of the endotoxin concentration of the sampled atmosphere.

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

1.5 It is anticipated that this practice will facilitate inter laboratory comparisons of airborne endotoxin data from metalworking fluid atmospheres, particularly metal removal fluid atmospheres, by providing a basis for endotoxin sampling, extraction, and analytical methods.

1.6 In 1997, the Occupational Safety and Health Administration (OSHA) empanelled a Standards Advisory Committee to make recommendations to the Administration regarding measures that the Administration could take to improve the health of workers exposed to metalworking fluids. A report to the Assistant Secretary of Labor for OSHA was submitted in July, 1999. Subcommittee E34.50 believes that the user community would benefit significantly if a standard method was developed to give the community guidance on a methodology for the sampling and analysis of personal airborne endotoxin exposure assessments in facilities using water-miscible metal removal fluids, based on the LAL assay or other endotoxin detection technologies as they become available.

1.7 This practice does not attempt to set or imply limits for personal exposure to endotoxin in metalworking fluid aerosols in workplace environments.

1.8 *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:²

D 1356 Terminology Relating to Sampling and Analysis of Atmospheres

D 4840 Guide for Sample Chain-of-Custody Procedures

D 5337 Practice for Flow Rate Calibration of Personal Sampling Pumps

D 6629 Guide for Selection of Methods for Estimating Soil Loss by Erosion

E 1370 Guide for Air Sampling Strategies for Worker and Workplace Protection

E 1497 Practice for Selection and Safe Use of Water-Miscible and Straight Oil Metal Removal Fluids

E 1542 Terminology Relating to Occupational Health and Safety

2.2 OSHA Standards:³

29 CFR 1910.1000 Air Contaminants

29 CFR 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories

2.3 Other Documents:

Criteria Document for a Recommended Standard: Occupational Exposure to Metalworking Fluids⁴

NIOSH Manual of Analytical Methods (NMAM)⁴

3. Terminology

3.1 For definitions of terms in this practice relating to sampling and analysis of atmospheres, refer to Terminology

² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

³ Available from Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

⁴ Available from U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Pkwy., Cincinnati, OH 45226.

¹ This practice 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.

Current edition approved April 1, 2007. Published June 2007. Originally approved in 2001. Last previous edition approved in 2001 as E 2144 - 01.



D 1356. For definitions of terms in this practice relating to occupational health and safety, refer to Terminology **E 1542**.

3.2 Definitions of Terms Specific to This Standard:

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

3.2.2 *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 3D 1 EU.

3.2.3 *field blank*, *n*—filter/cassette unit prepared for sampling that is taken to the sampling site and handled in the same manner as the analytical filter/cassette unit, but that is not a part of the sampling process.

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

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

3.2.6 *metal removal fluids*, *n*—the subset of metal working fluids that are used for wet machining or grinding to produce the finished part.

3.2.6.1 *Discussion*—The term most often refers to straight oils and water-based fluids, such as soluble, semi-synthetic, and synthetic fluids.

3.2.7 *onset time*, *n*—time required for a change of 200 mOD (optical density) units relative to the initial OD value.

3.2.8 *personal sampler*, *n*—a portable sampling instrument that is attached to a person to ascertain the concentration of specific constituents in the air in the person's breathing zone.

3.2.9 *pyrogen-free*, *adj*—material(s) devoid of measurable endotoxin activity.

3.2.10 *pyrogen-free water (PFW)*, *n*—processed water that is devoid of measurable endotoxin activity.

4. Summary of Practice

4.1 A known volume of workplace air in a facility utilizing metalworking fluids is drawn through a sample filter cassette unit.

4.2 The sample filter is extracted into a pyrogen-free solution to quantitatively release endotoxin absorbed from collected metalworking fluid aerosol.

4.3 The extract solution is subjected to quantitative endotoxin analysis techniques. The measured endotoxin concentration is reported in terms of endotoxin potency units per unit volume of air sampled.

5. Significance and Use

5.1 Endotoxins in metalworking fluid aerosols present potential respiratory health hazards to workers who inhale them. Therefore, a consensus standard is needed to provide reliable data on workplace airborne endotoxin concentrations where metalworking fluids are used.

5.2 This practice for measuring airborne endotoxin concentrations in metalworking fluid atmospheres will help to foster a better understanding of endotoxin exposure-response relationships.

5.3 This practice facilitates comparisons of inter laboratory data from methods and field investigative studies.

6. Interferences

6.1 Airborne endotoxin measurements resulting from use of LAL reagents are subject to inhibition/enhancement effects from a variety of bio-molecular species and physicochemical phenomena, such as pH, temperature, filter matrix effects, cationic concentrations, LAL-reactive materials (LRM), enzyme influences, and lysate composition variability and sensitivity (a function of different lysate processing methodologies).

7. Apparatus

7.1 Sampling:

7.1.1 *Sampling Unit*, an apparatus consisting of a personal sampling pump, a 37-mm glass fiber filter, a two-piece, closed-face plastic cassette, and flexible connecting tubing between the personal sampling pump and the attached cassette/filter unit.

7.1.1.1 *Pump*, a constant-flow personal sampling pump with an on-board battery power source and a flow rate of 2.0 L/min ($\pm 5\%$).

7.1.1.2 *Filter Cassette*, pyrogen-free, closed-faced, two-piece polystyrene filter holder with 4 mm inlet and outlet, with caps.

7.1.1.3 *Filter (Membrane)*, pyrogen-free, glass fiber, 37-mm diameter with a cellulose support pad.

7.1.1.4 *Connective Tubing*, flexible, appropriate inside diameter.

7.1.1.5 *Soap-bubble Meter*, a primary standard used for sampler flow rate calibration.

NOTE 1—An alternative primary standard is acceptable.

7.2 Extraction:

7.2.1 *Sonicator Bath*, ultrasonic/water bath apparatus with a minimum peak frequency of 40-kHz with cavitation adjustment and thermostat control.

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

8. Reagents and Materials

8.1 *Control Standard Endotoxin (CSE)*—Endotoxin preparations used for calibration standards shall be referenced to the Federal Drug Administration (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 directions.

9. Hazards

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

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

9.3 Inhalation or dermal exposure to metalworking fluids 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 personal protective equipment (see 29 CFR 1910.1000).

10. Pump Calibration and Standardization

10.1 Calibrate the airflow rate of the sampling pump on site before each sampling period. The final flow rate shall be determined after sample collection is complete. Samples should be voided if flow rate changes significantly during the sample period.

10.2 Maintenance and repairs of the sampling and analytical equipment should be performed according to the recommendations of the manufacturer and should be documented in maintenance records.

10.3 Airflow rate procedures shall be performed in accordance with Practice D 5337 or NMAM 1994 Calibration of Personal Sampling Pumps.

11. Sampling

11.1 Plan air-sampling strategies for metalworking fluid aerosol endotoxin analysis using Guide E 1370.

11.2 Filter/Cassette Unit Set-up:

11.2.1 Aseptically transfer a glass fiber filter and support pad to a closed-face, three-piece polystyrene cassette, and then assemble the cassette and seal the perimeter seams with PTFE tape.

11.2.2 Affix a label to the cassette with a unique sample identifier. The sample shall link to the following information: date of sample, location of work operation, sample volume, investigator/worker code, and any other pertinent information.

11.2.3 Store and transport at least one unused (blank) filter/cassette unit from the same lot as described in 11.2.1.

11.3 Personal Sampler:

11.3.1 Uncap filter/cassette unit and attach to calibrated personal sampler pump with flexible tubing.

11.3.2 Set the sampling rate of the personal sampling pump to 2.0 L/min ($\pm 5\%$) and record room temperature.

11.3.3 Attach the filter cassette in the breathing zone of the individual being tested, activate the personal sampling pump and record the starting time. Total sampling duration shall be determined on the basis of partial or total workday shifts or discrete work activities.

11.3.4 Deactivate the sampling pump after the sampling period and record the stopping time, temperature, and any unusual conditions in the sampling area that could bias the outcome of the sampling procedure.

11.3.5 Remove the used filter/cassette unit and cap at each end.

12. Storage and Shipment

12.1 Store the used labeled filter/cassette unit(s) in a suitable container at $4 \pm 2^\circ\text{C}$ until shipped or analyzed. Do not freeze sample at any time.

12.2 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 suitable container at $4 \pm 2^\circ\text{C}$.

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

12.4 Upon receiving the used filter/cassette unit(s), laboratory personnel shall record the date and time of receipt of the sample(s). Prior to extraction, samples shall be stored at $4 \pm 2^\circ\text{C}$. Do not freeze.

13. Extraction

13.1 The analyst shall subject sample and blank filters to extraction procedures on the same day that the filters are removed from their cassettes. Samples shall be allowed to warm to room temperature and the entire extraction procedure shall be conducted at room temperature ($25 \pm 2^\circ\text{C}$).

13.2 Aseptically remove the filter and support pad from the cassette with depyrogenated tweezers and place filter into a pyrogen-free test tube. Discard support pad.

13.3 Add an extraction volume of 20 mL of pyrogen-free water into the test tube which is then capped and bath sonicated at a minimum peak frequency of 40 kHz for 1 h at $25 \pm 2^\circ\text{C}$ (or shake vigorously for 1 h).

13.4 Centrifuge the extract at 1000 g for at least 15 min.

13.5 Transfer the supernatant into a pyrogen-free test tube.

13.6 Determine the pH of an aliquot of the sample extract, and if necessary, adjust the sample extract to pH 7.5 with pyrogen-free sodium hydroxide or hydrochloric acid.

14. Procedure

14.1 Analysis of sample extracts with endotoxin detection reagents shall be performed in accordance with manufacturer's directions.

15. Quality Assurance

15.1 Ensure validation and maintenance procedures have been conducted in accordance with spectrophotometer manufacturer's directions.

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 linearity of standard curve, spike recovery 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 kit manufacturer's directions.

16.2 Resultant endotoxin concentration data shall be expressed in endotoxin units per cubic metre of sampled air (EU/m^3).

16.3 Currently there is no data supporting a clinically significant exposure limit for airborne endotoxin.

17. Report

17.1 Report the following information as a minimum:

17.1.1 The purpose for which the test was conducted.

17.1.2 The location and date the sample was taken.

17.1.3 The airflow rate, collection time, and cubic metres of air collected.

17.1.4 The extraction method and type of filter used.

17.1.5 The concentration of endotoxin in the sample in EU per cubic metre of air.

17.1.6 If desired, a reference to levels of endotoxin in EU per cubic metre of air of an office area or other suitable area in the facility not routinely or never exposed to metalworking fluid mists.

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

17.1.7.1 LAL detection deviations as enhancements or spike recovery problems.

17.1.7.2 Method of air collection.

18. Keywords

18.1 aerosol sampling; airborne endotoxin; depyrogenation; endotoxin; endotoxin assay; endotoxin units (EU); extraction methods; Gram-negative bacteria; *Limulus* amoebocyte lysate (LAL) assay; metalworking fluid aerosols; metalworking fluids; metal removal fluids; sampling and analysis; workplace atmospheres

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