Designation: ~~E2657 – 16~~ E2657 – 21

An American National Standard

Standard Practice for Determination of Endotoxin Concentrations in Water-Miscible Metalworking Fluids[1]

This standard is issued under the fixed designation E2657; 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~~ Scope*

1.1 This practice covers quantitative methods for the sampling and determination of bacterial endotoxin concentrations in ~~water miscible~~ water-miscible metalworking fluids (MWF).

1.2 Users of this practice need to be familiar with the handling of MWF.

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

1.4 This practice replaces Method E2250.

1.5 This practice seeks to minimize ~~inter-laboratory~~interlaboratory variation of endotoxin data but does not ensure uniformity of results.

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~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D2881 Classification for Metalworking Fluids and Related Materials
D4840 Guide for Sample Chain-of-Custody Procedures
E1488 Guide for Statistical Procedures to Use in Developing and Applying Test Methods
E1497 Practice for Selection and Safe Use of Water-Miscible and Straight Oil Metal Removal Fluids
E1542 Terminology Relating to Occupational Health and Safety
E2250 Method for Determination of Endotoxin Concentration in Water Miscible Metal Working Fluids (Withdrawn 2008)[3]
E2523 Terminology for Metalworking Fluids and Operations

[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 ~~Oct. 1, 2016~~Nov. 1, 2021. Published ~~October 2016~~December 2021. Originally approved in 2009. Last previous edition approved in ~~2011~~2016 as ~~E2657 - 11.~~E2657 – 16. DOI: ~~10.1520/E2657-16.~~10.1520/E2657-21.

[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] The last approved version of this historical standard is referenced on www.astm.org.

***A Summary of Changes section appears at the end of this standard**

2.2 *Government Standard:*[4]
29 CFR 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories
2.3 *Other Documents:*[5]
NIOSH Criteria Document for a Recommended Standard: Occupational Exposure to Metalworking Fluids, 1998 ~~NIOSH~~
NIOSH Manual of Analytical Methods (NMAM), ~~4th ed., Eller and Cassinelli, Eds., 1994~~5th Edition

3. Terminology

~~3.1 For definitions of terms relating to this practice, refer to Terminology E1542.~~

3.1 ~~*Definitions of Terms Specific to This Standard:*~~*Definitions:*

3.1.1 For definitions of terms relating to this practice, refer to Terminologies E1542 and E2523.

3.1.2 *control standard endotoxin (CSE), n*—a purified preparation of endotoxin based on the USP Reference Standard Endotoxin (RSE); used in laboratories to prepare standard solutions.

3.1.3 *endotoxin, n*—pyrogenic high molar mass lipopolysaccharide (LPS) complex associated with the cell wall of ~~gram-negative~~Gram-negative bacteria.
3.1.3.1 *Discussion*—
Though endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a Limulus Amoebocyte Lysate (LAL) test.

3.1.4 *endotoxin unit (EU), n*—a biological potency unit equivalent to the FDA Reference Standard Endotoxin (RSE).
3.1.4.1 *Discussion*—
The current RSE (EC-6) is equivalent to ~~1ng~~ 1 ng = 10 EU.

~~3.2.4 *geometric mean (GM), n*—the central tendency of a set of numbers expressed as the nth root of their product.~~

~~3.2.5 *geometric standard deviation (GSD), n*—the spread of data in a set of numbers expressed as a geometric mean.~~

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

~~3.2.7 *inhibition/enhancement phenomenon, n*—conditions or artifacts in sample solutions that cause endotoxin concentration data from LAL assays to be less than or more than the concentration of endotoxin actually present in a given aqueous sample.~~

3.1.6 *Limulus amebocyte lysate (LAL) assay, n*—a biological assay dependent on a series of cascading enzyme reactions that occur when Limulus blood cell (amebocyte) lysate combines with endotoxin.

~~3.2.9 *metalworking fluid (MWF), n*—any fluid used for the purpose of cooling or treating metal surfaces during metal removal, metal forming or surface protection or preservation.~~

3.1.7 *metal removal fluid (MRF), n*—any fluid in the subclass of metalworking fluids used to ~~cut,~~cut or otherwise take away material or piece of stock.
3.1.7.1 *Discussion*—
Metal removal fluids include straight or neat oils ~~(~~(Classification D2881~~),~~) not intended for further dilution with water, and ~~water miscible~~ water-miscible soluble oils, ~~semisynthetics~~semi-synthetics, and synthetics, which are intended to be diluted with water before use. Metal removal fluids become contaminated during use in the workplace with a variety of workplace substances including, but not limited ~~to,~~to: abrasive particles, tramp oils, cleaners, dirt, metal fines and shavings, dissolved metal and hard water salts, bacteria, fungi, microbiological decay products, and waste. These contaminants can cause changes in the lubricity and cooling ability of the metal removal fluid as well as have the potential to adversely affect the health and welfare of employees in contact with the contaminated metal removal fluid.

[4] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.
[5] Available from CDC/NIOSH, 4676 Columbia Pkwy, Cincinnati, OH 45226-1998.

3.1.8 ~~*Operator-dependent assay,*~~ *metalworking fluid (MWF), n*—~~an assay performed by a technician in such a manner to cause significant influence(s) on the resultant data.~~any fluid used for the purpose of cooling or treating metal surfaces during metal removal, metal forming, or surface protection or preservation.

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

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

~~3.2.14 *sensitivity range, n*—a span of endotoxin measurements expressed as EU/mL or λ.~~

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *geometric mean (GM), n*—the central tendency of a set of numbers expressed as the nth root of their product.

3.2.2 *geometric standard deviation (GSD), n*—the spread of data in a set of numbers expressed as a geometric mean.

3.2.3 *inhibition/enhancement phenomenon, n*—conditions or artifacts in sample solutions that cause endotoxin concentration data from LAL assays to be less than or more than the concentration of endotoxin actually present in a given aqueous sample.

3.2.4 *operator-dependent assay, n*—an assay performed by a technician in such a manner to cause significant influence(s) on the resultant data.

3.2.5 *sensitivity range, n*—a span of endotoxin measurements expressed as EU/mL or λ.

4. Summary of Practice

4.1 Serial dilutions of CSE in PFW in borosilicate glass test tubes are prepared to construct a calibration curve.

4.2 The metalworking fluid sample is sonicated, centrifuged, and the supernatant retained.

4.3 Triplicates of the sample supernate, standard serial dilutions, blanks, and positive control solutions are subjected to the kinetic chromogenic LAL assay.

4.4 If data indicate interferences are present, MWF supernate is diluted and assay is performed with diluted supernate.

4.5 The reaction of Limulus amebocyte lysate with sample endotoxin imparts a proportional yellow color to the analyte solution that is measured photometrically at 405 nm.

4.6 The measured endotoxin concentration is reported as EU/mL.

5. Significance and Use

5.1 The determination of endotoxin concentrations in MWF is a parameter that can be used in decision-making for prudent fluid management practices (fluid draining, cleaning, recharging, or biocide dosages).

5.2 This standard provides a practice for analysts who perform quantitative endotoxin analyses of water-miscible MWF.

6. Interferences

6.1 Data from samples analyzed by LAL methodologies are prone to variations due to batch differences in lysate composition/processing, non-optimal pH, and temperatures of assay solutions.

6.2 In the event that the phenomenon of inhibition/enhancement influences this practice, endotoxin concentration data will be less than or more than actual concentrations present in a given MWF sample.

6.3 LAL assays are highly influenced by the skill/experience level of the analyst.

7. Apparatus

7.1 *Sampling:*

7.1.1 *Sample Collection Container,* pyrogen-free, wide-mouth, stainless steel sealable container, at least 100 mL capacity.

7.1.2 *Glass Pipet,* pyrogen-free, 50 mL.

7.1.3 *Battery-Powered Aspirator Unit (or suction bulb),* compatible with 100 mL glass pipet.

7.2 *Extraction:*

7.2.1 *Centrifuge,* minimum rotational speed of 5000 rpm.

7.2.2 *Ultrasonic Water ~~Bath,~~ Bath* ~~ultrasonic/water~~Ultrasonic/water bath apparatus with a minimum peak frequency of 40 kHz with cavitation adjustment and thermostat control; use pyrogen-free glass containers only.

7.3 *Analysis:*

7.3.1 *Incubating/Shaking Microplate Reader,* spectrophotometric at 405 nm.

7.3.2 *Statistical Analysis Software Package for Microplate Reader.*

7.3.3 *Vortexer,* variable speed.

7.3.4 *Microtiter Plates,* flat-bottomed, pyrogen-free, 96-well.

7.3.5 *Dilution Tubes,* pyrogen-free, 13 by 100 mm.

7.3.6 *Borosilicate Glass Test Tubes,* pyrogen-free, screw caps, 10 by 75 mm.

7.3.7 *Single-Channel Micropipetor(s),* ~~0.5-10~~ 0.5 to 10 µL.

7.3.8 *Eight-Channel Micropipetor,* 100 µL.

7.3.9 *Pipet Tips,* pyrogen-free, 300 µL.

7.3.10 *Glass Rod,* pyrogen-free.

7.3.11 *Reagent Reservoir,* pyrogen-free, 8-channel multipipettor compatible.

7.3.12 *Parafilm M.*

8. Reagents and Materials

8.1 *Control Standard Endotoxin (CSE),* referenced to most current Federal Drug Administration (FDA) Reference Standard Endotoxin (RSE).

8.2 *Limulus Amebocyte Lysate (LAL),* unexpired with stated potency.

8.3 *Dilution Water,* pyrogen-free ~~((~~PFW).~~PFW).~~

8.4 *MWF Concentrate,* concentrated, unused MWF as supplied.

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 Inhalation or dermal exposure to metalworking fluids pose potential health problems for personnel involved in MWF sampling. Provision of personal protective equipment (PPE) in the form of respirators or protective clothing, or both, is potentially indicated (see Practice E1497 and Criteria Document for a Recommended Standard: Occupational Exposure to Metalworking Fluids).

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

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 Procedure

10.1 *Sampling Site:*

10.1.1 Select sampling site that will yield a representative MWF sample.

10.1.2 Select individual sump(s) or central system(s) that has actively circulating fluids. If possible, draw sample from the ~~mid-point~~midpoint of the fluid reservoir. Otherwise, draw sample below the surface of the metalworking fluid volume of interest and avoid the aspiration of extraneous floating biomass.

10.1.3 Use aseptic techniques with pyrogen-free apparatus to aspirate a 100-mL grab sample with a glass pipet into a suitable pyrogen-free 250-mL container and then seal securely with a pyrogen-free lid or Parafilm M. Avoid touching inner lid and interior container areas with hands/gloves or nonpyrogenic labware.

11. Sample Storage/Shipment

11.1 For best results, LAL analysis of the sample within ~~24 hours~~ 24 h is advisable. However, if this is not feasible, store the sealed sample container in a plastic bag and then refrigerate or pack in crushed ice at 4 ± ~~2°C.~~2 °C. Avoid freezing sample, since this will adversely affect resultant data.

11.2 If the sample is shipped to an analytical laboratory, pack its container securely in cold packs (or portable refrigeration) and expedite shipment time so that the sample arrives at the laboratory no later than ~~24 hours~~ 24 h after its acquisition.

11.3 Maintain procedures for sample custody in accordance with accepted ~~chain of custody~~ chain-of-custody procedures (see Guide D4840).

12. Preparation of Labware

12.1 A critical consideration of quantitative LAL analyses is that the sample must be protected against the indiscriminate introduction of exogenous sources of endotoxin:

12.1.1 Commercially packaged labware used in LAL analyses shall be clearly marked as “pyrogen-free,” “endotoxin-free,” “depyrogenated,” or clearly identified as suitable for use in LAL analyses. A certificate of authentication shall accompany labware that attests to its pyrogen-free condition. Manufacturer ID, lot numbers, expiration dates, and authentication/certification information shall be recorded in laboratory notebooks.

12.1.2 Commercially packaged labware that is nominally described or labeled as “sterile,” “sterilized,” “disinfected,” or otherwise identified as suitable for routine microbiological usage only shall not be used in this standard practice, due to the possibility of the presence of residual endotoxin on critical labware surfaces.

12.1.3 Prior to use in this standard practice, non-pyrogen-free glass or metal labware that will be used in LAL analyses shall be