



Standard Practice for Enumeration of Non-Tuberculosis *Mycobacteria* in Aqueous Metalworking Fluids by Plate Count Method¹

This standard is issued under the fixed designation E2563; 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 the detection and enumeration of viable and culturable rapidly growing *Mycobacteria* (RGM), or non-tuberculosis *Mycobacteria* (NTM) in aqueous metalworking fluids (MWF) in the presence of high non-mycobacterial background population using standard microbiological culture methods.

1.2 The detection limit is one colony forming unit (CFU)/mL metalworking fluid.

1.3 This practice involves culture of organisms classified as Level 2 pathogens, and should be undertaken by a trained microbiologist in an appropriately equipped facility. The microbiologist should also be capable of distinguishing the diverse colonies of *Mycobacteria* from other microorganism colonies on a Petri dish and capable of confirming *Mycobacteria* by acid-fast staining method.

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

1.5 *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

[D5465 Practices for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods](#)

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, 2023. Published October 2023. Originally approved in 2007. Last previous edition approved in 2018 as E2563 – 18. DOI: 10.1520/E2563-23.

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.

[E1326 Guide for Evaluating Non-culture Microbiological Tests](#)

[E2523 Terminology for Metalworking Fluids and Operations](#)

2.2 *Other Document:*³

[Kinyoun Acid-Fast Staining Procedure](#)

3. Terminology

3.1 For definitions of terms used in this standard, refer to Terminology [E2523](#).

3.2 Definitions:

3.2.1 *rapidly growing mycobacteria (RGM)*—non-tuberculous *Mycobacteria* that grow and produce visible colonies in four to seven days.

4. Summary of Practice

4.1 For recovery and enumeration of viable and culturable *Mycobacteria* population in metalworking fluid field samples, selective culture medium containing antimicrobial agents to suppress bacterial and fungal contamination is recommended. (See Section 8.) Standard microbiological spread and droplet plating techniques are used for the enumeration of *Mycobacteria* (see Practices [D5465](#)). After a minimum of 14 days incubation at 30 °C, the *Mycobacteria* colonies are counted and confirmed by acid-fast staining technique specific for *Mycobacteria*.

5. Significance and Use

5.1 This practice allows for the recovery and enumeration of viable and culturable, non-tuberculosis, rapidly growing *Mycobacteria* (*M. immunogenum*, *M. chelonae*, *M. abscessus*, *M. fortuitum*, and *M. smegmatis*) in the presence of high Gram-negative background populations in metalworking fluid field samples. This population is predominantly comprised of Gram-negative bacteria and fungi. Mycobacterial contamination of metalworking fluids has been putatively associated with hypersensitivity pneumonitis (HP) amongst metalgrinding machinists. The detection and enumeration of these organisms will aid

³ *Public Health Microbiology: A Guide for the Level III Laboratory*, Centers for Disease Control, U.S. Department of Health and Human Services, Atlanta, GA, 1985.

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