



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

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

2. Referenced Documents

2.1 ASTM Standards:²

D5465 Practice for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods

E1326 Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria

2.2 Other Documents:³

Kinyuon Acid-Fast Staining Procedure

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.

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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 *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.

3. Terminology

3.1 Definitions:

3.1.1 *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*. 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. During the past decade it has become increasingly apparent that non-tuberculous *Mycobacteria* are common members of the indigenous MWF bacterial population. 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 metal grinding machinists. The detection and enumeration of these organisms will aid in better understanding of occupational health related problems and a better assessment of antimicrobial pesticide efficacy.

5.2 The measurement of viable and culturable mycobacterial densities combined with the total mycobacterial counts (including viable culturable (VC), viable-non culturable (VNC) and non viable (NV) counts) is usually the first step in establishing any possible relationship between *Mycobacteria* and occupational health concerns (for example, HP).

5.3 The practice can be employed in survey studies to characterize the viable-culturable mycobacterial population densities of metal working fluid field samples.