Designation: ~~E2563 – 13~~ E2563 – 18

An American National Standard

Standard Practice for Enumeration of Non-Tuberculosis *Mycobacteria* in Aqueous Metalworking Fluids by Plate Count Method[1]

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~~ acid-fast staining ~~method~~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~~ safety, health, and ~~health~~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

E1326 Guide for Evaluating Non-culture Microbiological Tests

E2523 Terminology for Metalworking Fluids and Operations

2.2 *Other ~~Documents:~~Document:*[3]

~~Kinyuon~~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

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

Section 8~~).~~.) Standard microbiological spread and droplet plating techniques are used for the enumeration of ~~*Mycobacteria.*~~*Mycobacteria* (see Practices D5465). After a minimum of 14 days incubation at ~~30°C,~~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. immunogenum,*~~*M.chelonae,*~~*M. chelonae,M. absessus,M. fortuitum*, and ~~*M.smegmatis*~~*M. smegmatis*) in the presence of high ~~gram negative~~ 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~~ Gram-negative bacteria and fungi. Mycobacterial contamination of metalworking fluids has been putatively associated with hypersensitivity pneumonitis (HP) amongst ~~metal grinding~~ metalgrinding machinists. The detection and enumeration of these organisms will aid in better understanding of occupational ~~health related~~ health-related problems and a better assessment of antimicrobial pesticide efficacy.

5.2 The measurement of viable and culturable mycobacterial densities (Guide E1326), combined with the total mycobacterial counts (including viable culturable (VC), ~~viable-non culturable~~viable nonculturable (VNC) and ~~non viable~~ nonviable (NV) ~~counts)~~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~~ metalworking fluid field samples.

5.4 This practice is also applicable for establishing the mycobacterial resistance of metalworking fluid formulations by determining mycobacterium survival by means of plate count technique.

5.5 This practice can also be used to evaluate the relative efficacy of microbicides against *Mycobacteria* in metalworking fluids.

6. Interferences

6.1 In some ~~metal working~~ metalworking fluid samples, very high (>10^6/mL) microbial background population ~~levels;~~levels, mainly ~~gram negative~~ Gram-negative pseudomonads and fungi, can interfere with the enumeration of *Mycobacteria* by "overgrowth" on the agar surface.

6.2 Sample dilution or smaller sample size can be used to minimize interference of non-target bacterial and fungal densities. Replicates of sample dilutions could be also plated and the results combined.

6.3 In some metalworking fluid samples, chemicals (antimicrobial pesticides, functional additives, and other components) can interfere with the culturability of total viable *Mycobacteria* count in the sample. If interference by chemicals is suspected, sample dilution may also overcome this interference but will reduce sensitivity.

7. Apparatus

7.1 *Laboratory Incubator,* ~~Laboratory Incubator,~~ 30 ± ~~2°C.~~2 °C.

7.2 *Microscope,* ~~Microscope~~ with oil immersion lens, magnification 1000×.

7.3 *Staining Tray or Sink,* ~~Staining tray or sink~~ with running water and drying rack.

8. Reagents and Materials

8.1 *Test Tubes,* with ~~close fitting~~ close-fitting or airtight caps, 20 by ~~150 mm,~~ 150 mm, sterile.

8.2 *Test Tube Racks,* sufficient size to hold 20 by ~~150-mm~~150 mm test tubes.

8.3 *Sterile Spreaders.*

8.4 *Sterile Loops.*

8.5 *Sterile ~~1-mL~~ 1 mL Pipets,* with ~~0.01-mL~~0.01 mL divisions.

8.6 *Dilution Water Blanks,* sterile, 9 mL.

8.7 *Selective Mitchison Modified 7H11 Agar.*

8.8 *Microscope Slides.*

8.9 *Paraffinic Laboratory Film,* 2.54 cm wide.

8.10 *Staining Reagents,* for ~~Acid Fast Staining~~ acid-fast staining procedure for staining *Mycobacteria* by the Kinyoun (cold) acid-fast procedure.

8.10.1 *TB Quick Stain Reagents:*

8.10.1.1 *Carbolfuchsin Reagent A*—Basic ~~Fuchsin~~fuchsin 17.0 g, ~~Aqueous Phenol~~aqueous phenol 1000 mL (aqueous solution of ~~Phenol~~phenol containing approximately 10 % water).