



Designation: D5465 – 16 (Reapproved 2020)

Standard Practices for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods¹

This standard is issued under the fixed designation D5465; 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 These practices cover recommended procedures for counting colonies and reporting colony-forming units (CFU) on membrane filters (MF) and standard pour and spread plates.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *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.*

1.4 *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

D1129 Terminology Relating to Water

D5259 Test Method for Isolation and Enumeration of Enterococci from Water by the Membrane Filter Procedure

D5392 Test Method for Isolation and Enumeration of *Escherichia coli* in Water by the Two-Step Membrane Filter Procedure

D6161 Terminology Used for Microfiltration, Ultrafiltration, Nanofiltration, and Reverse Osmosis Membrane Processes

D6974 Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures

E2563 Practice for Enumeration of Non-Tuberculosis *Mycobacteria* in Aqueous Metalworking Fluids by Plate Count Method

2.2 Other Standards:

APHA 9215 Heterotrophic Plate Count³

3. Terminology

3.1 Definitions:

3.1.1 For definitions of terms used in this standard, refer to Terminologies D1129 and D6161.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *colony forming unit (CFU)*, *n*—in microbiology, a visible mass of cells (algae, bacteria, or fungi) originating from either an individual cell or cluster of cells that have been placed onto or dispersed into a solid or semi-solid nutrient medium and subsequently incubated under prescribed conditions.

3.2.1.1 *Discussion*—Prescribed growth conditions can include, but are not limited to: growth medium pH and nutrient composition, incubation temperature, incubation environment (for example: gas mixture, pressure and relative humidity), and incubation interval. Any given set of growth conditions will select for the culture recovery of a fraction of a sample's microbiome and against the culture recovery of the balance of that microbiome.

3.2.1.2 *Discussion*—Recognizing that all culture test methods are selective, CFU data invariably underestimate the population densities of viable microbes in samples tested by those methods. Moreover, incomplete disaggregation of masses of microbial cells during sample preparation contributes to decreasing the ratio of CFU to total viable microbes in the sample.

3.2.1.3 *Discussion*—Colonies normally become visible to the naked eye only after approximately 1 billion cells have amassed. Assuming that the colony derived from a single cell, it requires approximately 30 generations for a single cell to proliferate to a mass of 1 billion cells. Consequently, the time lapse between inoculation and detection of a CFU is directly dependent on the generation time(s) of taxa present in sample. Moreover, because colony diameter increases as the cells

¹ These practices are under the jurisdiction of ASTM Committee D19 on Water and are the direct responsibility of Subcommittee D19.24 on Water Microbiology.

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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 Available from American Public Health Association (APHA), 800 I St., NW, Washington, DC 20001, <http://www.apha.org>.