



Standard Test Method for Producing High Titers of Viable and Semi-Purified Spores of *Clostridium difficile* using a Liquid Medium¹

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INTRODUCTION

Clostridium difficile (*C. difficile*), an anaerobic spore-former, can cause acute and potentially fatal gastroenteritis in healthcare and other settings (1).² The frequent episodes of diarrhea can contaminate the indoor environment widely with persistent (2) and microbicide-resistant (3) spores. Disinfectants wishing to claim activity against *C. difficile* now require carrier testing using spores of high purity (>90%) that show a >6 log₁₀ reduction in spore viability (4). While the use of a semi-solid medium for *C. difficile* spore production has been reported (5) (Test Method E2839), this standard describes a liquid medium and an enzyme-based semipurification process for the purpose. Appendix X1 describes an alternative to enzyme purification.

1. Scope

1.1 This test method describes the production and semipurification of *C. difficile* spores (also called endospores) primarily for use in testing the sporicidal activities of environmental surface disinfectants (Test Methods E2111 and E2197); such spores can also be used to study their structure, chemistry and germination.

1.2 While the test method described is based on the use of 500-mL volumes of the liquid culture medium in an anaerobic incubator, anaerobic jars with smaller volumes of the same medium can also be used.

1.3 It is the responsibility of the investigator to determine whether Good Laboratory Practice (GLP) regulations are required and to follow them when appropriate (40 CFR, Part 160 for EPA submissions and 21 CFR; Part 58 for FDA submissions).

1.4 **Warning**—This standard may involve hazardous materials, chemicals, and microorganisms and should be performed only by persons with formal training in microbiology.

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

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

2. Referenced Documents

2.1 ASTM Standards:³

D1129 Terminology Relating to Water

E2111 Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemicals

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E2839 Test Method for Production of *Clostridium difficile* Spores for Use in Efficacy Evaluation of Antimicrobial Agents

2.2 Federal Standards:⁴

21 CFR, Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies

40 CFR, Part 160 Good Laboratory Practice Standards

¹ This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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² The boldface numbers in parentheses refer to a list of references at the end of this standard.

³ 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.

⁴ Available from Superintendent of Documents, U.S.S Government Printing Office, Washington DC, 20402. <http://www.gpo.gov/>