



Designation: E3011 – 22

Standard Practice for In vitro production of *Clostridioides difficile* Spores¹

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1. Scope

1.1 This practice is designed to propagate spores of *Clostridioides difficile* using liver broth.

1.2 It is the responsibility of the user of this practice to determine whether Good Laboratory Practices are required and follow when appropriate.

1.3 This practice should only be performed by those trained in microbiological techniques.

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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.*

1.6 *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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Federal Standard*:³

40 CFR, Part 160 Good Laboratory Practice Standards

¹ This practice 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 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 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) 40 CFR Part 160, Good 185 Laboratory Practice Standards; Final Rule. 1989.

3. Terminology

3.1 *Definitions*: For definitions of general terms used in this practice, refer to Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard*:

3.2.1 *frozen stock culture, n*—a culture of vegetative bacteria propagated, and prepared for storage at $\leq -70^{\circ}\text{C}$ in a liquid broth medium containing a cryoprotectant such as glycerol.

3.2.2 *spore suspension, n*—harvested spores suspended in a liquid medium, sterile deionized water.

4. Summary of Practice

4.1 This standard outlines a procedure for producing high-titer spore suspensions of *C. difficile* using a commercially available liquid medium with 7 d to 10 d of incubation under anaerobic conditions. Once adequate levels of spores are present in the liquid medium, the spores are harvested and washed several times in cold sterile deionized water. The spore suspension is enumerated, the spore purity is assessed and the acid resistance is verified.

5. Significance and Use

5.1 This practice describes a procedure for producing spore suspensions of *C. difficile* ATCC 700792, *C. difficile* ATCC 43598, or *C. difficile* ATCC 43599. The spore suspensions may be used in antimicrobial efficacy testing, or other laboratory testing requiring *C. difficile* spores. A spore crop is considered acceptable if the titer is $>8 \log_{10}$ spores/mL, purity of 95 %, and is resistant to 2.5M HCl after 10 min of exposure.

6. Apparatus

6.1 *Anaerobe jar*—Any airtight jar or container that can be used in combination with gas packs to obtain an anaerobic environment. An anaerobic chamber and anaerobic incubator may be substituted for an anaerobe jar.

6.2 *Biological safety cabinet*—To help maintain an aseptic work space.

6.3 *Centrifuge*—Any type or model capable of centrifuging up to 40 mL of liquid at $7500 \times g$.

6.4 *Centrifuge tubes*—Any type of sterile centrifuge tube with a 50 mL capacity.

6.5 *Incubator*—An incubator capable of maintaining $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.