



Standard Practice for Characterization of *Bacillus* Spore Suspensions for Reference Materials¹

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INTRODUCTION

Bacillus spp. are aerobic, rod-shaped, Gram positive bacteria that produce endospores under nutrient limiting conditions. The endospores are designed to persist in extreme environments and consequently are highly resistant to inactivation by heat, chemicals and irradiation. A few species of *Bacillus* are medically important because of their impact on human and animal health while others have important agricultural and industrial applications. Measurement of viable *Bacillus* spores present in a suspension can be performed using classical microbiology techniques, such as growth on nutrient medium. The spore suspension is diluted, an aliquot spread on solid nutrient medium, incubated at an appropriate temperature, and the resulting colonies counted. The selection of the type of growth medium and incubation temperature for the optimal growth of a particular *Bacillus* species should be determined by consultation of relevant literature or by comparison of different growth media and incubation temperatures.

Bacillus spore reference materials have many important applications in agriculture, basic research, medical diagnosis, detector validation, and sterility testing. Uniform methods for the characterization of spores will improve the comparison of different lots of materials and results between different laboratories.

1. Scope

1.1 This practice is focused on two basic measurements to characterize *Bacillus* reference materials, the enumeration of spores using growth of colonies on nutrient media and using phase contrast microscopy to determine spore quality and homogeneity. Additional information on advanced methods for characterization is provided in [Appendix X1](#).

1.2 This document will provide the user with recommendations for measurement methods, and the details and conditions that should be employed to ensure reliable and high-quality data are obtained. The practice will help ensure that results obtained from the characterization are reported in a uniform manner. This will allow others to replicate the measurements and facilitate the comparison of different lots of *Bacillus* spore suspensions used as reference materials. It is important to note that the *Bacillus* species are a heterogeneous group and their specific requirements for growth and sporulation may vary.

Users of this practice are encouraged to consult the literature for specific information on the species of *Bacillus* bacteria they are using **(1)**.²

1.3 This standard practice does not provide guidance for the identification of unknown species of bacteria. The identification of *Bacillus* species has been traditionally based on colony morphology, growth on selective media, and biochemical tests, but more recently nucleic acid technologies have enabled the phylogenetic analysis of this group based on 16S DNA sequence similarities **(1)**.

1.4 Some *Bacillus* spp. are pathogenic to humans and animals and the user is advised to adhere to safe laboratory procedures and practices for handling spores from these species **(2)**. 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)**.

1.5 This practice assumes a basic knowledge of microbiology and molecular biology and access to the cited references.

¹ This practice is under the jurisdiction of ASTM Committee E54 on Homeland Security Applications and is the direct responsibility of Subcommittee E54.01 on CBRNE Sensors and Detectors.

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