Journal of Microbiological Methods 92 (2013) 375–380

Contents lists available at SciVerse ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

A rapid and repeatable method to deposit bioaerosols on material surfaces

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ARTICLE INFO

Article history:
Received 20 December 2012
Received in revised form 14 January 2013
Accepted 14 January 2013
Available online 4 February 2013

Keywords:
Aerosol deposition
Surface sampling
Anthrax
Bacillus anthracis
Biological agent

ABSTRACT

A simple method for repeatably inoculating surfaces with a precise quantity of aerosolized spores was developed. Laboratory studies were conducted to evaluate the variability of the method within and between experiments, the spatial distribution of spore deposition, the applicability of the method to complex surface types, and the relationship between material surface roughness and spore recoveries. Surface concentrations, as estimated by recoveries from wetted-wipe sampling, were between 5×10^3 and 1.5×10^4 CFU cm^{-2} across the entire area (930 cm^2) inoculated. Between-test variability (C_v) in spore recoveries was 40%, 81%, 66%, and 20% for stainless steel, concrete, wood, and drywall, respectively. Within-test variability was lower, and did not exceed 33%, 47%, 52%, and 20% for these materials. The data demonstrate that this method is repeatable, is effective at depositing spores across a target surface area, and can be used to dose complex materials such as concrete, wood, and drywall. In addition, the data demonstrate that surface sampling recoveries vary by material type, and this variability can partially be explained by the material surface roughness index. This deposition method was developed for use in biological agent detection, sampling, and decontamination studies, however, is potentially beneficial to any scientific discipline that investigates surfaces containing aerosol-borne particles.

Published by Elsevier B.V.

1. Introduction

In the years following the anthrax letter attacks of 2001, the amount of research conducted on bioterrorism-related issues has increased significantly (AOAC International, 2011). The consensus among many experts is that the sampling and analysis methods used to detect, collect, and quantify contamination should be better characterized, enhanced, and validated (Sanderson et al., 2002; U.S. Government Accountability Office, 2005). While numerous studies have been conducted in the past decade to further our understanding of currently-accepted sampling methods (Hodges et al., 2010; Hodges et al., 2006; Krauter et al., 2012; Probst et al., 2011; Rose et al., 2011), only a few have utilized aerosol-deposited bacterial spores to inoculate surfaces (Brown et al., 2007a, 2007c; Edmonds et al., 2009; Estill et al., 2009). The experience following the 2001 letter attacks suggests that *Bacillus anthracis* contamination spread by aerosol is highly likely following an incident (Dull et al., 2002; Fennelly et al., 2004; Krauter and Biermann, 2007; Weis et al., 2002). However, the specialized equipment and expertise necessary to generate precise and accurate aerosol concentrations within deposition chambers prevents the wide-spread use of this method to inoculate test materials. Recently, a method for conducting aerosol inoculations was developed (Lee et al., 2011), however the current method is limited to inoculation of small (18–25 mm-diameter) surface areas. The purpose of this paper is to describe a novel method for depositing aerosolized *Bacillus* spores onto material surfaces for the purposes of detection, sampling, and decontamination research studies. This paper describes a repeatable and rapid method of surface inoculation that is realistic of aerosol-derived contamination mechanisms, such as an aerosol dissemination of *B. anthracis* spores. This method does not require sophisticated aerosol deposition chambers, and is therefore not restricted with regards to the size, shape, and surface area of the materials to be inoculated.

2. Materials and methods

2.1. Aerosol deposition apparatus

The aerosol deposition apparatus (ADA) is a pyramid-shaped chamber designed for use with a pressurized and metered dose dispenser. The chamber shape and dimensions (Fig. 1A) were designed to accommodate near-complete (>99.99% in number) deposition of particles within 18 h, based upon deposition via gravitational settling of 1 μm aerodynamic diameter particles (Tuladhar et al., 2012). The chamber was constructed of stainless steel (16-gauge, 304 stainless; Dillon Supply, Raleigh, NC) so that it could be easily sterilized between uses. Four ports located near the base of the pyramid were outfitted with 0.2 μm pore-size syringe filters (Millipore, Billerica, MA) to allow pressure equilibrium during dosing, yet prevent infiltration of contaminants or exfiltration of the test organism. The top of the pyramid contained a 3.3 cm diameter orifice for the introduction of the aerosol dose. An 11.4 by 4.7 by 1.3 cm sliding lid, also equipped with a 3.3 cm diameter orifice, facilitated coupling of the dose dispensing device and sealing of

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0167-7012/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.mimet.2013.01.015

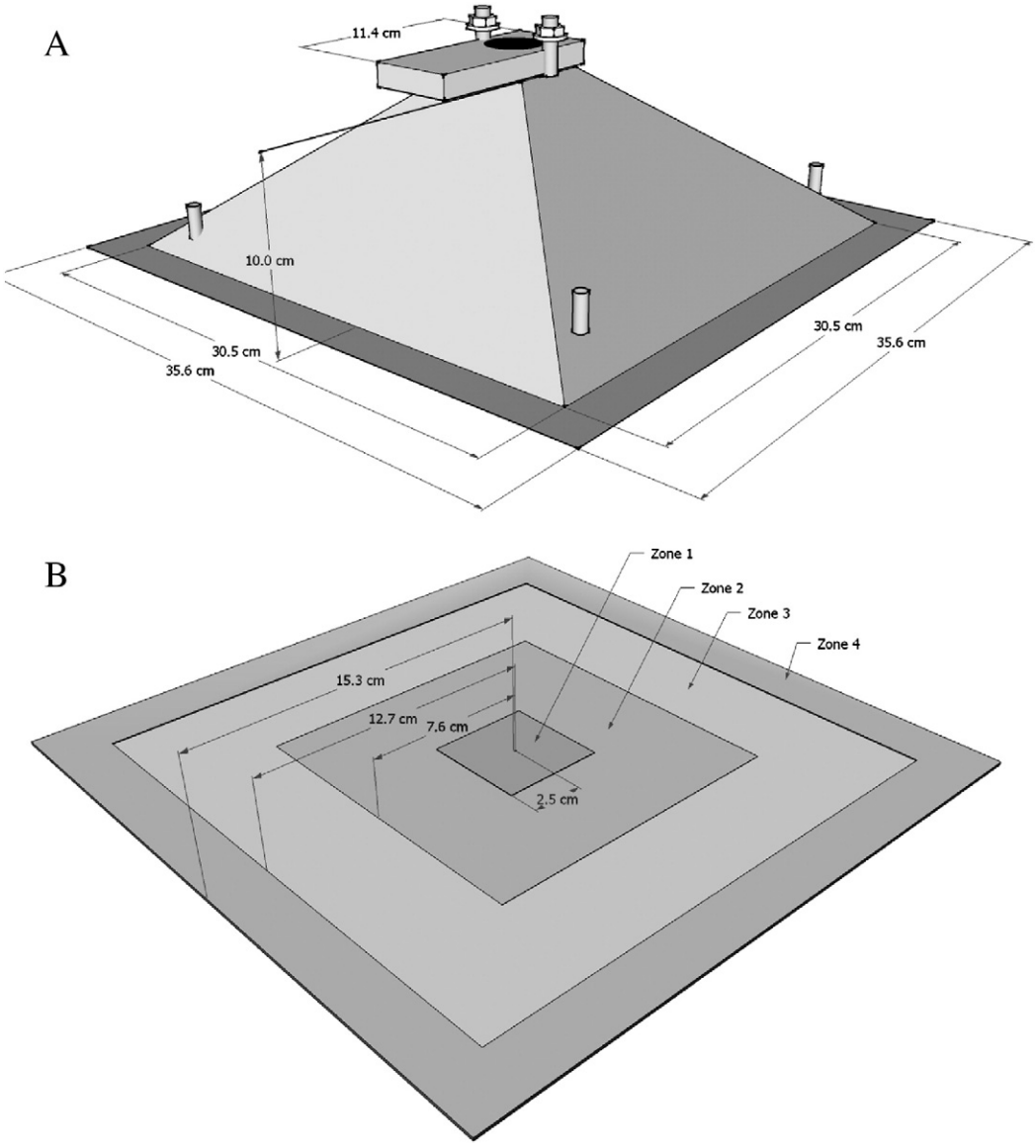

Fig. 1. Drawings of the aerosol deposition apparatus (A) and segmented stainless steel coupon utilized to evaluate the spatial distribution of the spore deposition (B).

the ADA prior to and following the dispensing of the aerosol dose. The interior dimensions of the ADA, and thus the area dosed by this device, are 30.5 by 30.5 cm. The base of the ADA contains a 2.54 cm-wide flange, which aids in sealing the ADA to the target material's surface and results in an overall footprint of 35.6 by 35.6 cm (Fig. 1A). The design of the apparatus allows controlled dosing of precise areas on large objects without the size restriction and investment required by aerosol test chambers.

2.2. Preparation of material coupons

Stainless steel (16-gauge, 304 stainless; Dillon Supply, Raleigh, NC) was used as a representative non-porous smooth surface material, and was cut into 35.6 cm by 35.6 cm coupons from larger pieces of stock material (Fig. 2A). To determine the spatial distribution of the spore inoculum over the 30 by 30 cm area, three stainless steel coupons were sectioned into four interlocking concentric squares (Fig. 1B). The inner-most section (5 by 5 cm square, Zone 1) was positioned at the center of the 30 by 30 cm area. Moving outward from the center section, was a 15.25 by 15.25 cm section (Zone 2) surrounded by a 25.4 by 25.4 cm section (Zone 3), which is also surrounded by a 30.6 by 30.6 section (Zone 4). Zones 1, 2, 3, and 4 correspond to: zero to 2.5 cm, 2.5 to 7.6 cm, 7.6 to 12.7 cm, and 12.7 to 15.3 cm distance from the coupon center, respectively.

Concrete, wood, and drywall coupons were utilized as representative indoor and outdoor surfaces (Fig. 2B–E). Concrete coupons were prepared by mixing Quikrete® Sand/Topping Mix (Atlanta, GA, USA) according to the manufacturer's instructions and pouring into 35.6 by 35.6 by 3.81 cm moulds. Surfaces were smoothed by a hand trowel and allowed to cure for at least 5 days under plastic sheeting. Prior to use in tests, loose grit was removed from the concrete surfaces by spraying them with water using a gas-powered pressure washer.

Wood coupons were prepared by cutting 35.6 cm by 35.6 cm coupons from larger pieces of pressure-treated plywood (alkaline copper quaternary type D, 1.91 cm thick, Georgia-Pacific, Atlanta GA), or by assembling three pieces of (two 35.6 by 13.7 by 1.3 cm pieces, one 35.6 by 8.5 by 1.3 cm piece) pressure-treated Brazilian Pine Dog Ear Picket Fence lumber (Product# 884-831, Home Depot, Durham, NC) into one 35.6 cm by 35.6 cm coupon.

Drywall coupons were prepared by cutting 35.6 cm by 35.6 cm coupons from 1.22 m by 2.44 m by 1.27 cm size pieces of gypsum drywall (Part# 258-350, Home Depot, Durham, NC). To increase the