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technical BRIEF
BUILDING A SCIENTIFIC FOUNDATION FOR SOUND ENVIRONMENTAL DECISIONS

Sampling Procedures Using Commercially Available Robotic Floor Cleaners for *Bacillus anthracis* Spores

INTRODUCTION

Figure 1.
Example of robotic floor cleaners (RFCs).

Following the 2001 intentional contamination incidents involving *Bacillus anthracis* in the United States (DOJ 2010), the research need for sampling methods for the remediation of contaminated buildings became evident. Subsequently, surface sampling studies were emphasized due to the direct impact that sampling results have on decontamination decision making. Even though there have been improvements in methods and procedures for real-world applications, significant improvement in sampling and analysis processes in the event of a biological attack on a wide urban area is still needed. The currently recommended surface sampling approaches require the use of varied methods that can be both labor intensive and expensive to implement. Research indicates that commercially available robotic floor cleaners (RFCs, Figure 1) offer a safe, efficient, and economical option in addition to currently-used surface sampling methods.

Results (Figure 2) from previous laboratory studies demonstrate sampling (collection) using RFCs can be as efficacious on porous and nonporous surfaces as presently recommended methods such as the use of swabs, wipes, and vacuums fitted with filter-type collection media typically used on small discrete areas. RFCs use mechanisms for surface sampling similar to the methods applied in traditional processes such as vacuuming, sweeping, and scrubbing. Many RFCs have functions and sensors allowing for enhanced cleaning performance and operation in multiple rooms on one battery charge. Such a procedure used in *B. anthracis* spore sampling would permit fewer samples compared

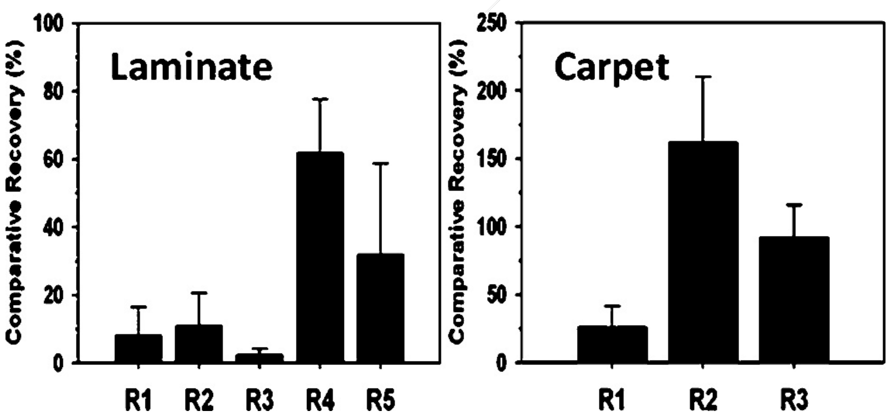

Figure 2.
Recovery of bacterial spores by robotic floor cleaners compared to currently recommended surface sampling methods. Five different RFCs were tested for laminate and three for carpet. R1, R2, R3: dry vacuum based RFC; R4: mopping RFC; R5: wet vacuum RFC

to currently used sampling methods, thereby lessening the burden placed on laboratories. Due to their autonomy, the robots expand the ability to sample limited-access and highly contaminated areas. There is also a potential for a reduction in personnel needs, for less time in the contaminated areas, and, most importantly, for lowered exposure risks for responders.

The sampling procedures that have been developed detail the step-by-step process for sampling a horizontal surface (e.g., floor) using an RFC. An equipment description and supply list describe the materials needed for sampling and how to secure the RFC unit for shipment, storage, and analysis. In the event of a biological contamination incident, specific pre-deployment preparations must be made to prepare the RFC for sample collection. After sample collection, handling guidelines and documentation instructions preserve the sample and address quality assurance requirements.

The RFC sampling procedure was used to sample a mock-up of a concrete subway station during a recent field study (EPA 2017). The results clearly showed that the RFC sampling method provides the benefits of reduced sampling time during a response, fewer samples requiring processing, detection of spore presence at unknown hot spots of contamination, improved detection of widespread contamination when concentrations are close to (or potentially below) detection limits for traditional surface sampling methods, and shortened timeline to response and recovery. In addition, the field study results confirmed the following cautions for using these methods:

- The tested composite sampling methods generated a large quantity of debris/dust in the sampling media. It is necessary to develop efficient sample processing procedures prior to analysis either at the site or at the laboratory.
- It was difficult to assess the actual sampled area due to the unpredictable movement of the robots. RFCs may stop sampling due to high filter pressure drop on dusty surfaces. It is recommended that a magnetic strip be used to pre-define a discrete sampling area (~100-200 ft^2, dependent upon the amount of floor debris present).

SCOPE AND APPLICABILITY

The sampling procedures have been developed by the U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program within EPA's Office of Research and Development jointly with the Chemical, Biological, Radiological and Nuclear Consequence Management Advisory Division within EPA's Office of Land and Emergency Management. The procedures are intended to guide trained incident-responders step-by-step to collect environmental samples following a biological contamination incident. The procedures are applicable to the collection of surface-bound particulates and microorganisms using off–the-shelf RFCs following a contamination incident. The purpose of the procedures is to guide the process of preparation, deployment, and collection using RFCs for sampling surfaces from a specified area. The collected samples will be used to determine presence/absence and/or contamination level after natural outbreaks and intentional or accidental releases of pathogenic microorganisms and biotoxins

At the date of this publication, the RFC sampling procedures have been partially characterized for deployment feasibility and collection performance for bacterial spores. At the time of publication, these procedures have not been validated. The Draft procedures will be updated or replaced with a fully characterized and validated sampling procedures upon availability. During emergency situations, the use of non-validated methods can be warranted where validated methods are not available. EPA's use of non-validated methods must adhere to the EPA's Forum on Environmental Measurement (FEM) policy directive on method validation (EPA 2010). Further information on method validation can be found in *Validation of U.S. Environmental Protection Agency Environmental Sampling Techniques that Support the Detection and Recovery of Microorganisms* (EPA 2012).

ADDITIONAL STUDIES

In 2013, U.S. EPA staff published two studies examining RFCs for surface sampling. In the first study published (Lee et al. 2013), testing was conducted using five commercially available robots on various indoor flooring surface materials. The purpose of this experiment was to determine how robotic floor cleaners (RFCs) used for sampling of *B. atrophaeus* spores (a surrogate for *B. anthracis*) compare to the currently recommended surface sampling methods. Some of the robotic cleaners performed as well as the respective floor sampling methods currently in use. The second published study (U.S. EPA 2013) evaluates the efficacy of commercially available, off-the-shelf RFCs as an alternative sampling technique for surfaces contaminated with *Bacillus* spores. The robots were tested on porous and nonporous surfaces and were evaluated in terms of efficiency, availability, and cost. The highest performing RFCs from this set were further tested at different levels of contamination. By comparing recoveries (of colony forming units), sampling via some RFCs was found to be as efficacious as other sampling methodologies currently recommended.

In Lee et al. (2014), commercially available vacuum-based and wetted-wipe-based robot cleaners were tested at different spore loading levels. The sampling ability of the RFC was evaluated in both hot spot and widely dispersed contamination scenarios. Cleaning robots were found to be capable of sampling as effectively as the currently used methods at different spore loading levels. The robots can improve the detection capability and cover a larger area than currently recommended sampling methods.

The EPA's recent report (U.S. EPA 2017) evaluates the field use of commercially available, off-the-shelf RFCs for surfaces contaminated with *Bacillus* spores as well as other composite sampling methods. The robots were tested on concrete platform surfaces at a mock subway station and were evaluated for operational challenges under dusty field conditions.

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