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DEVELOPMENT OF A ONE-HANDED, ENVIRONMENTAL SURFACE-SAMPLING DEVICE

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PREFACE

The work described in this report was authorized by the U.S. Army Edgewood Chemical Biological Center as a Section 219 Innovative Proposal Program for fiscal year 2015. The work was started in February 2015 and completed in October 2015.

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This report has been approved for public release.

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DEVELOPMENT OF A ONE-HANDED, ENVIRONMENTAL SURFACE-SAMPLING DEVICE

1. INTRODUCTION

At the time of this report, there were several biological surface-sampling kits that were chosen to acquire and safely transport biological specimens (1). In these kits, many of the items were individually packaged and required both hands for optimal use. Because of this individual packaging, an operator can generate a large amount of waste that needs to be managed during a sampling mission. The U.S. Army Edgewood Chemical Biological Center (ECBC), Research and Technology Directorate Biodefense Branch has designed a sampling device to facilitate biological sampling using one hand and reducing waste materials, which we have named the Mano Environmental Surface-Sampling Device (also referred to as the “Mano sampling device”). The advantage of this device as compared with existing sampling technology is that this device acts as the actual sampler as well as the transport packaging. This could provide a significant advantage for individuals collecting biological samples while wearing mission-oriented protective posture gear in a hazardous environment. This design indicates a major step forward in biological-sampling technology. In this report, we compared the effectiveness of the Mano sampling device to the biological sampling kit (BiSKit; QuickSilver Analytix, Inc.; Abingdon, MD), which was employed by the Department of the Army for large-area surface sampling at the time of this study (2). Results from this study demonstrated that the Mano sampling device was more efficient than the BiSKit in the collection of *Bacillus anthracis* simulants. Overall, our results indicated that the Mano sampling device should be further evaluated to include different surface materials, various microorganisms (e.g., Gram-negative bacteria and viruses), and toxins.

2. MATERIALS AND METHODS

2.1 Design for Manufacturing and Test Kit Production

The current Mano sampling device design was evaluated for manufacturability, and an outer sanitary container and packaging were added to ensure that sampling surfaces were not compromised (Figure 1). ECBC’s Advanced Design and Manufacturing team produced 25 sampling kits, which enabled the collection of 125 samples.



Figure 1. Mano Environmental Surface-Sampling Device.

2.2 Test Organism and Culture Media

The Gram-positive organism, *Bacillus atrophaeus* var. *globigii* (Bg; Unified Culture Collection designation: BACI051) was selected as a surrogate for the well-known biothreat agent, *Bacillus anthracis* Ames. This bacterial stock was obtained from the Critical Reagents Program (CRP; Frederick, MD). For surface spotting, Bg spores were diluted with Butterfield's buffer into 10^7 or 10^5 colony-forming units (cfu)/mL aliquots. Butterfield's buffer was prepared in accordance with the U.S. Food and Drug Administration's recommended preparation for food science research (3). For this study, Bg was cultured on tryptic soy agar plates (TSA) at pH 7.0 (Difco Laboratories, Inc.; Detroit, MI) and incubated at 37 °C for 18–24 h.

2.3 Preparation of Sampling Surfaces

For this study, we examined glass and stainless steel surfaces. Glass surfaces were 12 × 12 in., 0.25 in. thick industrial glass. The stainless steel surfaces were also 12 × 12 in. Both types of surfaces were initially cleaned with 70% ethanol to remove possible dust and/or dirt that may have accumulated during the manufacturing process. Next, the surfaces were autoclaved for 30 min at 121 °C, followed by a 10 min drying cycle. After the autoclave process, all samples were prepared and spore spotting was performed in a biological safety cabinet. For the spore-spotting procedures, the surfaces were spotted with 1 mL of either 10^7 or 10^5 cfu/mL of Bg spores and allowed to dry for at least 1 h.

2.4 Surface-Sampling Method

For these studies, we performed biological sampling using both BiSKit and Mano sampling devices on 12 × 12 in. surfaces according to the BiSKit User's Manual (Quicksilver Analytics) (4). Before sampling, each of the devices was hydrated with 15 mL of sterile 0.01 M potassium phosphate buffer with 0.05% Tween 20 (w/v) at pH 7.0. Although this buffer was supplied in the BiSKit complete kit, it was made up for the Mano sampling device in-house, and the materials needed were obtained from Sigma-Aldrich Company (St. Louis, MO). After hydration, the sampling devices were initially used in a horizontal overlapping "S" pattern, which was followed by a vertical "S" pattern. The buffer was then removed from the sampling device, collected in a 50 mL conical tube, and vortexed for 1 min.

2.5 Experimental Controls

Several controls are needed for surface-sampling studies. Blank samples consisted of sampling buffer alone. The titer controls (positive controls) consisted of 15 mL of collection buffer that was spiked with either 10^7 or 10^5 cfu of Bg spores. The negative controls were the hydrated sampling devices (either BiSKit or Mano sampling devices) that were not used for any sampling. Finally, we determined the maximum recovery efficiencies for the BiSKit and Mano sampling devices by spotting with Bg spores (10^7 or 10^5 cfu) and then finishing the collection procedure.

2.6 Live Culture Evaluation of the Sample-Collection Devices

After the samples were collected and vortexed, 100 μ L of collected buffer was plated on TSA plates using a cell spreader and turntable. The plates were then placed in the incubator at 37 °C overnight, and the resultant colonies were manually counted. The results were then expressed as the mean \pm standard deviation (SD) of the \log_{10} of recovered colony-forming units as well as the mean \pm SD of the percentage of recovered colony-forming units.

2.7 DNA Extraction

DNA extraction and purification were performed using the UltraClean Microbial DNA Isolation kit (MoBio Laboratories, Inc.; Carlsbad, CA) in accordance with the manufacturer's recommended protocol (5).

2.8 DNA Analysis

Extracted DNA was measured for quantity and quality using the Thermo Fisher Scientific NanoDrop spectrophotometer model 2000c (Waltham, MA) in accordance with the manufacturer's recommended protocol. Briefly, the NanoDrop instrument was blanked with TE buffer (Tris and ethylenediaminetetraacetic acid [EDTA]) before each measurement. Absorbance measurements for each sample were recorded in triplicate at 260 nm (A_{260}) and 280 nm (A_{280}). To determine the DNA concentration for each sample, the NanoDrop software used a modified Beer-Lambert equation and reported results in nanograms per microliter. To determine the purity of collected DNA, the ratio between A_{260} and A_{280} was calculated. An absorbance ratio of 1.8–2.0 was considered to be contaminant-free DNA. For both quantity and quality determinations, results were reported as the mean \pm SD of either the resultant nanograms per microliter calculations or the A_{260}/A_{280} ratios.

2.9 Quantitative Polymerase Chain Reaction (qPCR)

Extracted DNA samples were run for polymerase chain reaction (PCR) amplification in an ABI 7900HT Sequence Detection system (Life Technologies; Carlsbad, CA). Experimental samples were run at a concentration of 0.02 ng/ μ L, and 5 μ L of DNA sample was added to 15 μ L of master mix. The samples were then cycled in two stages: Stage 1 consisted of 1 cycle at 50 °C for 2 min and 1 cycle at 95 °C for 20 s. Stage 2 consisted of 45 cycles at 95 °C for 1 s, followed by 1 cycle at 60 °C for 20 s. The 6-carboxyfluorescein (FAM)/

tetramethylrhodamine (TAMRA)-labeled primers for BACI051 were obtained through the CRP and used as directed. The qPCR results for BACI051 were considered to be positive if the cycle threshold (Ct) value was <40, and results were considered to be negative if Ct values were >40 or absent. Results were reported as the mean \pm SD of resultant Ct values.

3. RESULTS

3.1 Maximum Recovery Efficiencies for the BiSKit and Mano Sampling Devices

To experimentally determine the maximum recovery efficiencies of the sampling devices, we added either 10^5 or 10^7 cfu of Bg spores directly to each of the collection devices and then processed the samples as described in Sections 2.4–2.9. The efficiencies shown for 10^5 cfu (Figure 2A) and 10^7 cfu (Figure 2B) displayed comparable results; Bg spores could be effectively released from both the BiSKit and the Mano sampling devices.

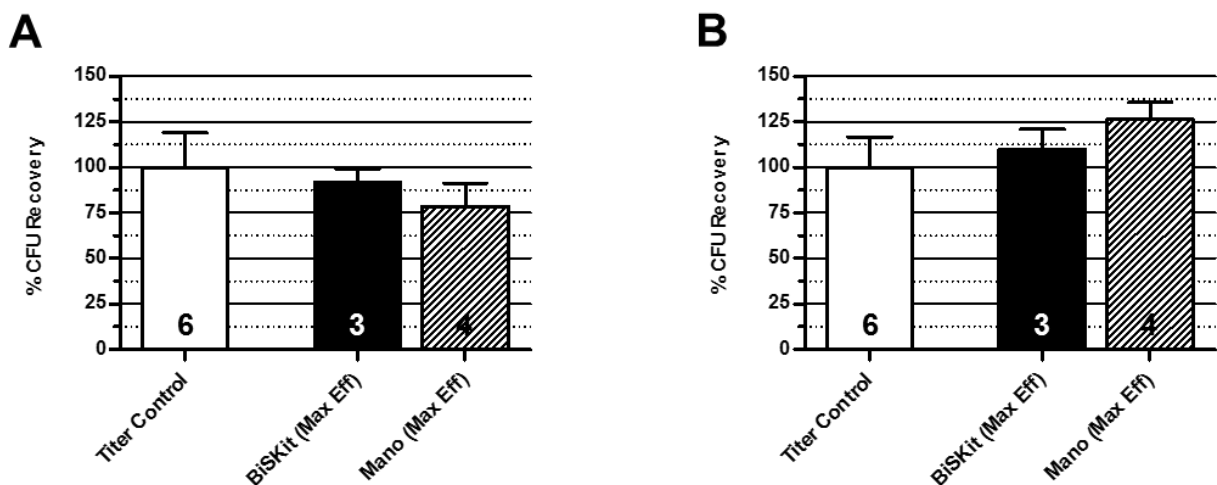


Figure 2. Maximum recovery efficiencies. Maximum recovery efficiencies for the BiSKit or Mano sampling devices spiked with (A) 10^7 cfu or (B) 10^5 cfu of Bg spores. Results are expressed as mean \pm SD of the percentage of recovered colony-forming units compared with titer control. The number of experiments (n) is located within each vertical bar.

3.2 Collection Efficiency for the BiSKit and Mano Sampling Devices on Glass Surfaces at 10^7 cfu of Bg Spores

The surface recovery of Bg spores on a glass surface was performed in accordance with the procedures described in Sections 2.3–2.9. Figure 3 shows the surface recovery of 10^7 cfu of Bg spores obtained using either the BiSKit or the Mano sampling devices. The results are reported as either the \log_{10} of recovered colony-forming units (Figure 3A) or the percentage of recovered colony-forming units (Figure 3B) as compared with titer controls. On this surface and at this spore concentration, the Mano sampling device recovered ~52% of the

spotted Bg spores. Under the same conditions, the BiSKit sampling device recovered ~34% of the spotted Bg spores.

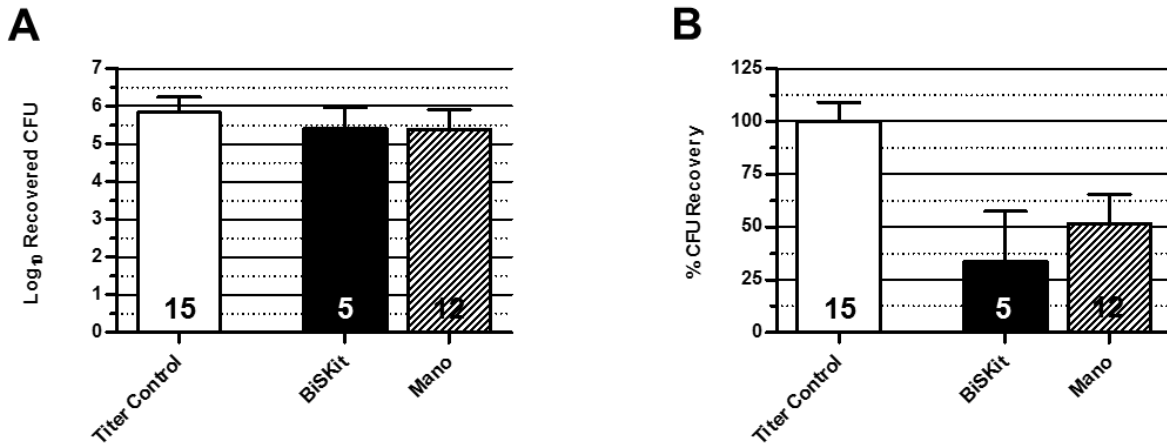


Figure 3. Collection efficiencies from glass at 10^7 cfu of Bg spores. Each 12×12 in. glass surface was inoculated with 10^7 cfu of Bg spores. The spores were then recovered from the sampling devices as described in Section 2. Results are expressed as mean \pm SD; (A) the \log_{10} of recovered colony-forming units; or (B) the percentage of recovered colony-forming units compared with titer control. The number of experiments (n) is located within each vertical bar.

3.3 Collection Efficiency for the BiSKit and Mano Sampling Devices on Stainless Steel Surfaces at 10^7 cfu of Bg Spores

The surface recovery of Bg spores on a stainless steel surface was performed according to the procedures described in Sections 2.3–2.9. Figure 4 shows the surface recovery of 10^7 cfu of Bg spores obtained using either the BiSKit or the Mano sampling devices. The results are reported as either \log_{10} of recovered colony-forming units (Figure 4A) or the percentage of recovered colony-forming units (Figure 4B) as compared with titer controls. On this surface and at this spore concentration, the Mano sampling device recovered ~24% of the spotted Bg spores. Under these same conditions, the BiSKit sampling device recovered ~26% of the spotted Bg spores.

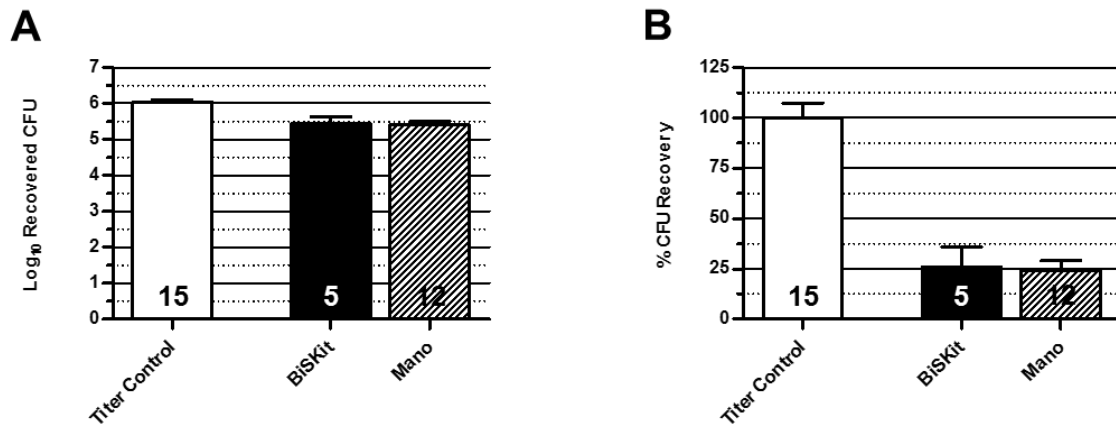


Figure 4. Collection efficiencies from stainless steel at 10^7 cfu of Bg spores. Each 12×12 in. stainless steel surface was inoculated with 10^7 cfu of Bg spores. The spores were then recovered from the sampling devices as described in Section 2. Results are expressed as mean \pm SD; (A) the \log_{10} of recovered colony-forming units; or (B) the percentage of recovered colony-forming units compared with titer control. The number of experiments (n) is located within each vertical bar.

3.4 Collection Efficiency for the BiSKit and Mano Sampling Devices on Glass Surfaces at 10^5 cfu of Bg Spores

The surface recovery of Bg spores on a glass surface was performed in accordance with the procedures described in Sections 2.3–2.9. Figure 5 shows the surface recovery of 10^5 cfu of Bg spores obtained using either the BiSKit or the Mano sampling devices. The results are reported as either \log_{10} of recovered colony-forming units (Figure 5A) or the percentage of recovered colony-forming units (Figure 5B) as compared with titer controls. On this surface and at this spore concentration, the Mano sampling device recovered ~49% of the spotted Bg spores. Under these same conditions, the BiSKit sampling device recovered ~29% of the spotted Bg spores.

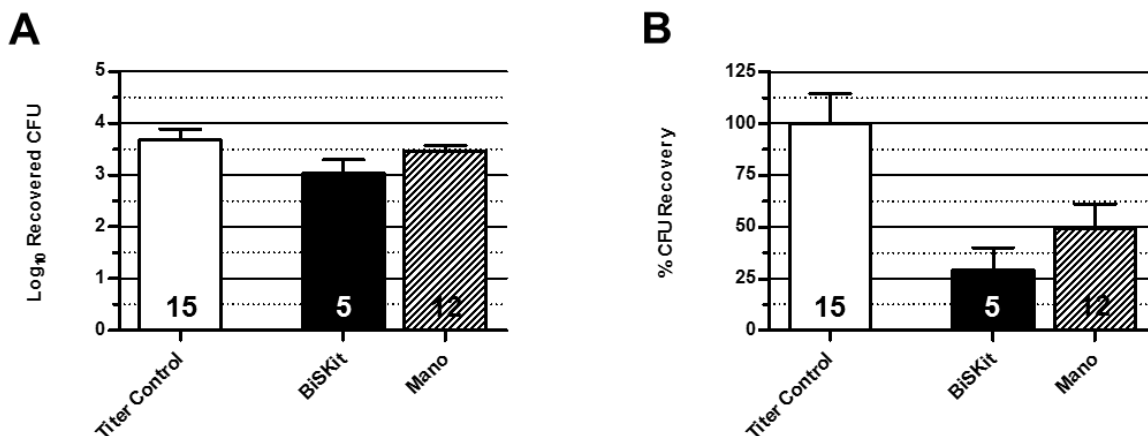


Figure 5. Collection efficiencies from glass at 10^5 cfu of Bg spores. Each 12×12 in. glass surface was inoculated with 10^5 cfu of Bg spores. The spores were then recovered from the sampling devices as described in Section 2. Results are expressed as mean \pm SD; (A) the \log_{10} of recovered colony-forming units; or (B) the percentage of recovered colony-forming units compared with titer control. The number of experiments (n) is located within each vertical bar.

3.5 Collection Efficiency for the BiSKit and Mano Sampling Devices on Stainless Steel Surfaces at 10^5 cfu of Bg Spores

The surface recovery of Bg spores on a stainless steel surface was performed in accordance with the procedure described in Sections 2.3–2.9. Figure 6 shows the surface recovery of 10^5 cfu of Bg spores obtained using either the BiSKit or the Mano sampling devices. The results are reported as either \log_{10} of recovered colony-forming units (Figure 6A) or the percentage of recovered colony-forming units (Figure 6B) as compared with titer controls. On this surface and at this spore concentration, the Mano sampling device recovered ~40% of the spotted Bg spores. Under these same conditions, the BiSKit sampling device recovered ~25% of the spotted Bg spores.

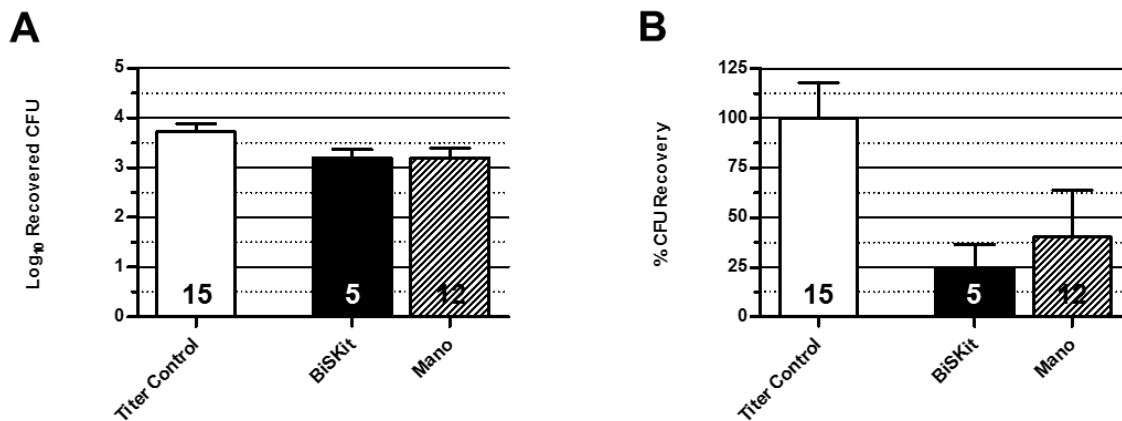


Figure 6. Collection efficiencies from stainless steel at 10^5 cfu of Bg spores. Each 12×12 in. stainless steel surface was inoculated with 10^5 cfu of Bg spores. The spores were then recovered from the sampling devices as described in Section 2. Results are expressed as mean \pm SD; (A) the \log_{10} of recovered colony-forming units; or (B) the percentage of recovered colony-forming units as compared with titer control. The number of experiments (n) is located within each vertical bar.

3.6 Quantity and Quality of DNA Recovered from the BiSKit and Mano Sampling Devices

DNA quantity results after the collection of Bg spores at 10^7 cfu from either glass or stainless steel surfaces are shown in Figure 7. All of the recoveries at this titer (10^7 cfu) produced a sufficient amount of nucleic acid to perform several PCR-based detection assays.

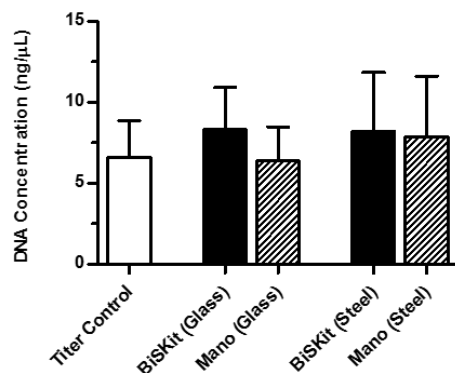


Figure 7. Quantities of recovered DNA at 10^7 cfu of Bg spores. Vertical bars represent the mean \pm SD of the recovered DNA concentration (ng/ μ L). The number of experiments (n) is located within each vertical bar.

DNA quality results after the collection of Bg spores at 10^7 cfu from either glass or stainless steel surfaces are shown in Figure 8. The recovered and purified DNA produced positive results when analyzed using PCR (Figure 8).

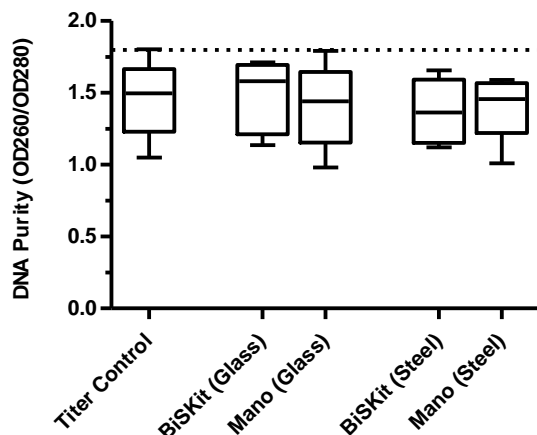


Figure 8. Quality of recovered DNA at 10^7 cfu Bg spores. Results are expressed as box-and-whisker plots of the mean \pm SD of the A_{260}/A_{280} ratio. The dotted line represents an A_{260}/A_{280} ratio of 1.8.

3.7 qPCR Results for the BiSKit and Mano Sampling Devices

We performed qPCR reactions on the resultant DNA after sample collections were obtained from glass and stainless steel using the CRP-validated Bg spore assay described in Section 2. Results are shown in Table 1. Results were similar for both the BiSKit and Mano sampling devices. These results indicated that the Mano sampling device or the buffer used for collection did not interfere with the qPCR detection of a specific target.

Table 1. qPCR Results

Test	Ct Values					
	10 ⁵ cfu of Bg Spores	Glass (10 ⁵ cfu of Bg Spores)	Steel (10 ⁵ cfu of Bg Spores)	10 ⁷ cfu of Bg Spores	Glass (10 ⁷ cfu of Bg Spores)	Steel (10 ⁷ cfu of Bg Spores)
Titer control	33.30 \pm 2.46	—	—	28.29 \pm 2.58	—	—
BiSKit	34.97 \pm 0.74	37.69 \pm 1.73	38.53 \pm 1.75	25.17 \pm 2.36	29.35 \pm 2.52	28.75 \pm 2.18
Mano	37.53 \pm 2.01	35.44 \pm 2.72	35.62 \pm 2.00	27.82 \pm 1.43	27.23 \pm 1.01	29.72 \pm 1.72

Notes: Nucleic acid extraction and qPCR were performed as described in Section 2. Results are displayed as the mean \pm SD of the reported Ct values. —, no data.

4. CONCLUSIONS

The initial results of this study indicate that in a laboratory setting, the Mano sampling device is capable of collecting a *B. anthracis* simulant with greater efficiency than the BiSKit device, which was in use by the Department of the Army at the time of this report. Also, this study demonstrates that the Mano device is capable of collecting samples that are compatible with currently used PCR technologies. Overall, these results indicate that the Mano sampling device should be evaluated further, and future studies should include a larger sample size, different surface materials, various microorganisms (e.g., Gram-negative bacteria and viruses), and toxins. These results could be used to create a new reference method for environmental surface sampling. As part of this study, the authors have submitted a Patent Disclosure to the U.S. Army Research, Development and Engineering Command, Office of the Chief Council (DAM824-14).

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ACRONYMS AND ABBREVIATIONS

BiSKit	biological sampling kit
Bg	<i>Bacillus atrophaeus</i> var. <i>globigii</i>
cfu	colony-forming units
CRP	Critical Reagents Program
Ct	cycle threshold
ECBC	U.S. Army Edgewood Chemical Biological Center
EDTA	ethylenediaminetetraacetic acid
FAM	6-carboxyfluorescein
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
SD	standard deviation
TAMRA	tetramethylrhodamine
TE buffer	Tris and ethylenediaminetetraacetic acid
TSA	tryptic soy agar

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