Evaluation of Analytical Methods for the Detection of *Bacillus anthracis* Spores: Compatibility with Real-World Samples Collected from Outdoor and Subway Surfaces

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The EPA is responsible for the remediation of land and public infrastructure following a biological contamination incident such as an act of bioterrorism involving the release of *Bacillus anthracis* (*B. anthracis*), the bacterium that causes anthrax, in an urban area. The EPA, in partnership with New York City and Battelle, investigated the impact of real-world interferents collected from mid-town Manhattan (Times Square and Grand Central Station areas) using Sponge-Sticks and vacuum filter cassettes (VFCs). Surface samples collected in the field were sent to the analytical laboratory, spiked with *B. anthracis* Sterne spores, then recovered and analyzed using Rapid Viability (RV) PCR and microbiological culture analytical methods developed by the EPA. From Sponge-Sticks, RV-PCR analytical method correctly detected the presence of viable *B. anthracis* in > 97% of spiked samples. By comparison, microbiological culture using Tryptic Soy Agar II (TSA with 5% Sheep Blood) correctly detected the presence of viable *B. anthracis* in 77% of spiked Sponge-Sticks, meaning the presence of real-world material collected during surface sampling can hinder identification of viable spores using the culture method. Neither the RV-PCR nor culture analytical methods performed as well with surface samples collected using the VFC method. Only 47% and 54% of spiked samples correctly identified as containing viable *B. anthracis* spores for RV-PCR and culture (all spike levels pooled), respectively. The relatively low positive identification success was attributed to poor physical recovery of *B. anthracis* Sterne spores from the VFC. The results from this study show that RV-PCR can be used to positively identify viable *B. anthracis* in presence of complex, dirty sample matrices from Sponge-Stick surface samples. The background flora and grime collected on the Sponge-Sticks can hinder detection and/or suppress the sensitivity of the *B. anthracis* signal, but samples with as few as 15 *B. anthracis* spores applied to the sponge could routinely be positively identified.