Evaluation of Pre- and Post-Sample Compositing for Low Concentration Bacillus spores from a simulated post-decontamination sampling of indoor surfaces

Background

Post-decontamination sampling is a potential step to confirm the effectiveness of decontamination efforts following a biological contamination incident. With the currently available standard sampling methods, which typically utilize small discrete area sampling and analysis protocols, post-decontamination sampling of large areas can be lengthy and present a high financial burden to responsible agencies. Furthermore, the wipe-based surface sampling methodologies typically focus on sampling of smooth and non-porous surfaces, and do not address porous environmental substrates. Composite sample collection and composite sample analysis can be a good complement to standard methods, and offer multiple potential advantages such as reduced response time (especially during widespread contamination of large area sampling), higher sample throughput, and lower analytical cost.

Aims of the study: (1) Evaluate the effectiveness of composite sample collection and composite sample analysis methods for quantitative determination of low surface concentrations (< 5000 CFU %) of Bacillus spores from representative semi-porous and porous indoor surfaces; (2) Perform the comparative analysis of operational parameters and environmental burden for various sampling methods.

Analytical methods

After sampling (Fig 2 a) samples were analyzed quantitatively for the number of viable spores recovered per sample (CFU). Briefly, sponge stick(s) were transferred to a sterile stomacher bag and extracted with 100 mL PBS (Fig 2b). After extraction (Fig. 2c through f), samples were filtered (Fig. 2 g to h), and incubated at 30 ± 2°C for 20-22 hours prior to manual enumeration (Fig 2i).

Experimental approach

• Target organism: Bacillus thuringiensis subsp. kurstaki (Btk): surrogate for Bacillus anthracis
• Test materials: Painted drywall and glazed ceramic tile; porous and semi-porous; inoculation control was stainless steel, coupon size 12 x 12 cm
• Surface concentrations: 50, 500 and 5000 spores per coupon delivered via direct liquid spike technique; first- and last-coupon contamination configurations were studied for single- and multi-pass composite sampling methods (SSC and SMC)
• Sampling test: Cellulose sponge stick pre-moistened with neutralizing buffer
• Sampled area: from 1 x 10 in to 10 in (single-location sampling) to 4 x 10 in to 10 in (multi-location sampling)

Composite analysis

Testing of standard and modified sampling protocols

- Single-location sampling (reference)
- Multi-location composite sampling

Testing of composite-sample analysis approaches

- Multi-sample extractions
- Operational time
- Amount of waste generated

Comparative analysis of operational parameters and environmental burden

Design of multivariate testing of sampling approaches

Efficacy of sample-collection compositing

The current methodology recommended for sampling non-porous smooth surfaces – which utilizes a single implement (spunge stick) for a multi-pass sampling of one discrete location (SMS method) offered the highest average recovery of target organism in all test material-surface concentration combinations tested (Fig 3). The average recovery (%Rec) for 1-point discrete area SMS sampling of semi-porous material (63 ± 11%; Fig. 3 a) was approximately 2 x %Rec for porous material (30 ± 10%; Fig. 3 b)

Conclusions

- SMS (or reference CDC method) offers the highest average recovery of target organism for all (semi-porous and porous) surface type and concentration combinations tested
- Both multi-location composite methods (SSC, SMC) showed decreases in target organism recoveries when compared to SMS, with multi-pass sampling generally outperforming the single-pass approach
- Contaminant transfers and diminishing collection efficiencies during sampling of consecutive areas within a four-point composite were observed for both SSSC and SMS
- Both SSSC and SMC methods offer the largest reduction of the total operational time and cost, and have lowest waste generation rates among all compositing approaches tested
- Post-sample compositing of multiple implements from a SMS sampling offers a balance between the analytical method performance and the time and cost of analysis
- Combination of composite sample collection approaches with composite sample analysis may offer the most savings on cost time, and at the expense of detection sensitivity

Operational time and waste generation

The comparative analysis of laboratory labor (normalized per area sampled) showed that both pre- and post-sample collection compositional approaches improve the overall operational time of the method (Fig 6). The waste generation metric exhibits a similar trend, with lowest waste generation rates recorded for SMC and SSC (0.15-0.16 lb per ft² sampled), followed by SPSC (0.31 lb per ft² sampled), and SMS (0.63 lb per ft² sampled).