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

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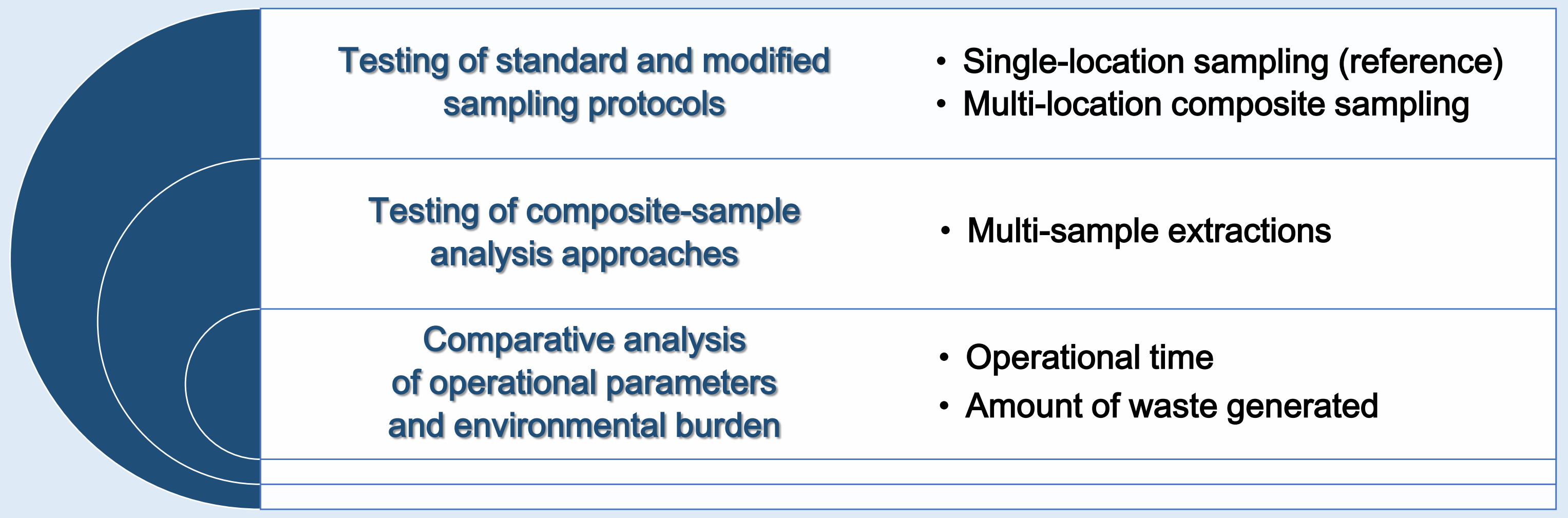
Background

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 or 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 ft^{-2}) of *Bacillus* spores from representative porous and semi-porous indoor surfaces; (2) Perform the comparative analysis of operational parameters and environmental burden for various sampling methods.



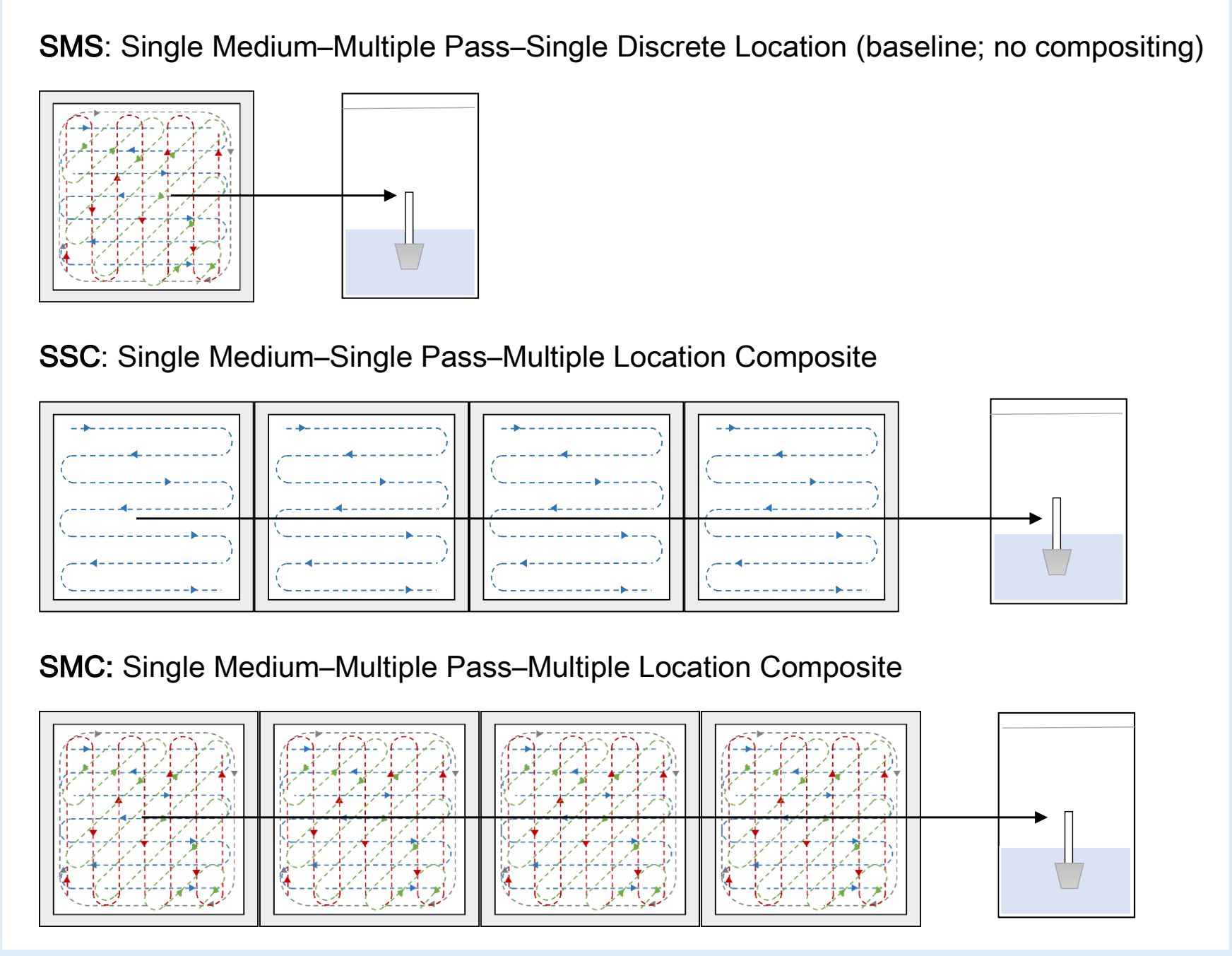
Testing of standard and modified sampling protocols	• Single-location sampling (reference) • Multi-location composite sampling
Testing of composite-sample analysis approaches	• Multi-sample extractions
Comparative analysis of operational parameters and environmental burden	• Operational time • Amount of waste generated

Figure 1 Design of multivariate testing of sampling approaches

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-in × 12-in
- **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 tool:** cellulose sponge stick pre-moistened with neutralizing buffer
- **Sampled area:** from 1 × 10-in × 10-in (single-location sampling) to 4 × 10-in × 10-in (multi-location sampling)

Composite sampling

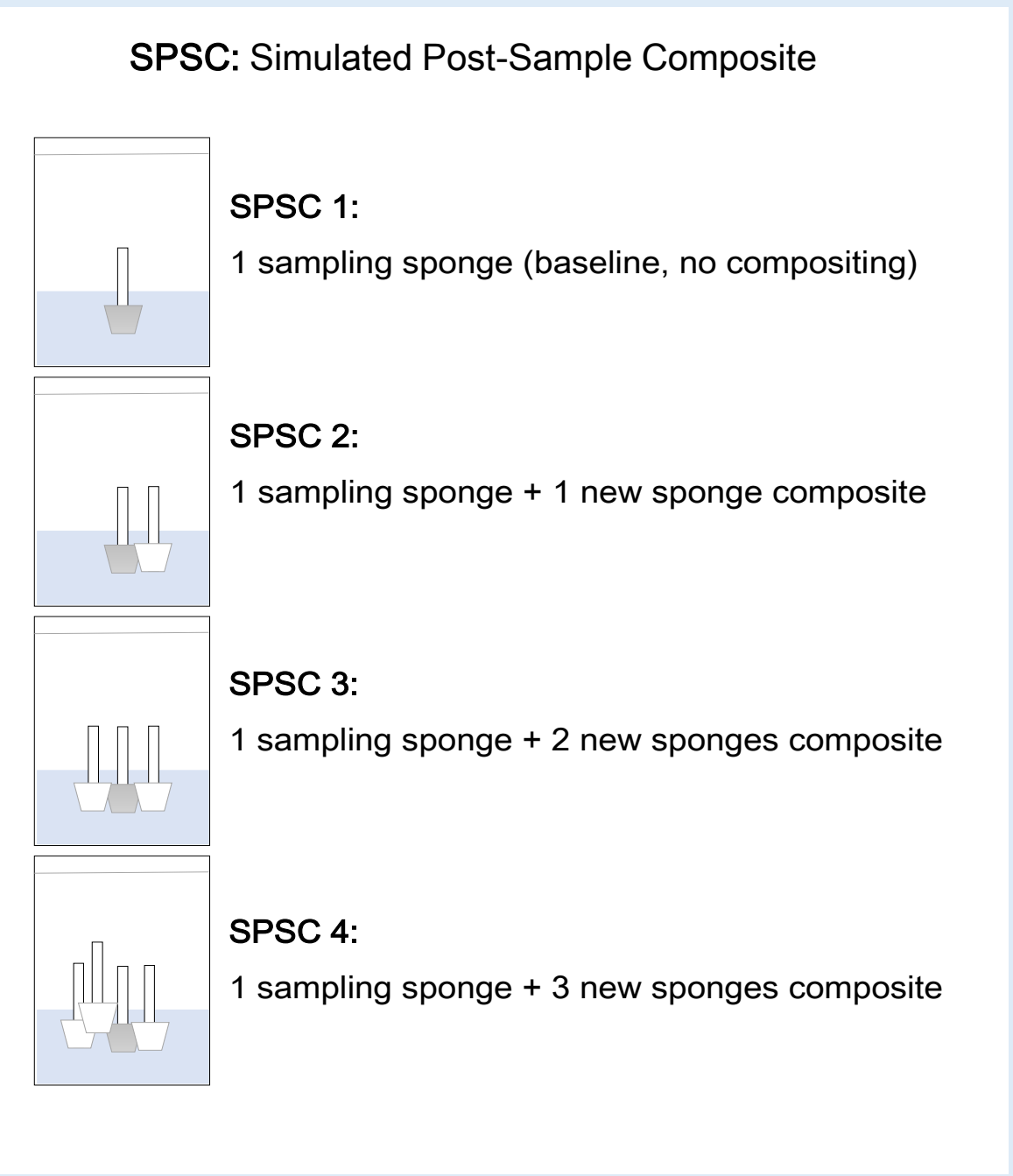


SMS: Single Medium–Multiple Pass–Single Discrete Location (baseline; no compositing)

SSC: Single Medium–Single Pass–Multiple Location Composite

SMC: Single Medium–Multiple Pass–Multiple Location Composite

Composite analysis



SPSC: Simulated Post-Sample Composite

SPSC 1: 1 sampling sponge (baseline, no compositing)

SPSC 2: 1 sampling sponge + 1 new sponge composite

SPSC 3: 1 sampling sponge + 2 new sponges composite

SPSC 4: 1 sampling sponge + 3 new sponges composite

SMS is a reference method from Centers for Disease Control and Prevention, CDC (2012) Surface sampling procedures for Bacillus anthracis spores from smooth, non-porous surfaces, https://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html;

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 PBST (Fig 2b). After extraction (Fig. 2c through f) samples were filter plated (Fig 2. g to h), and incubated at 30 ± 2 °C for 20–22 hours prior to manual enumeration (Fig 2 i).

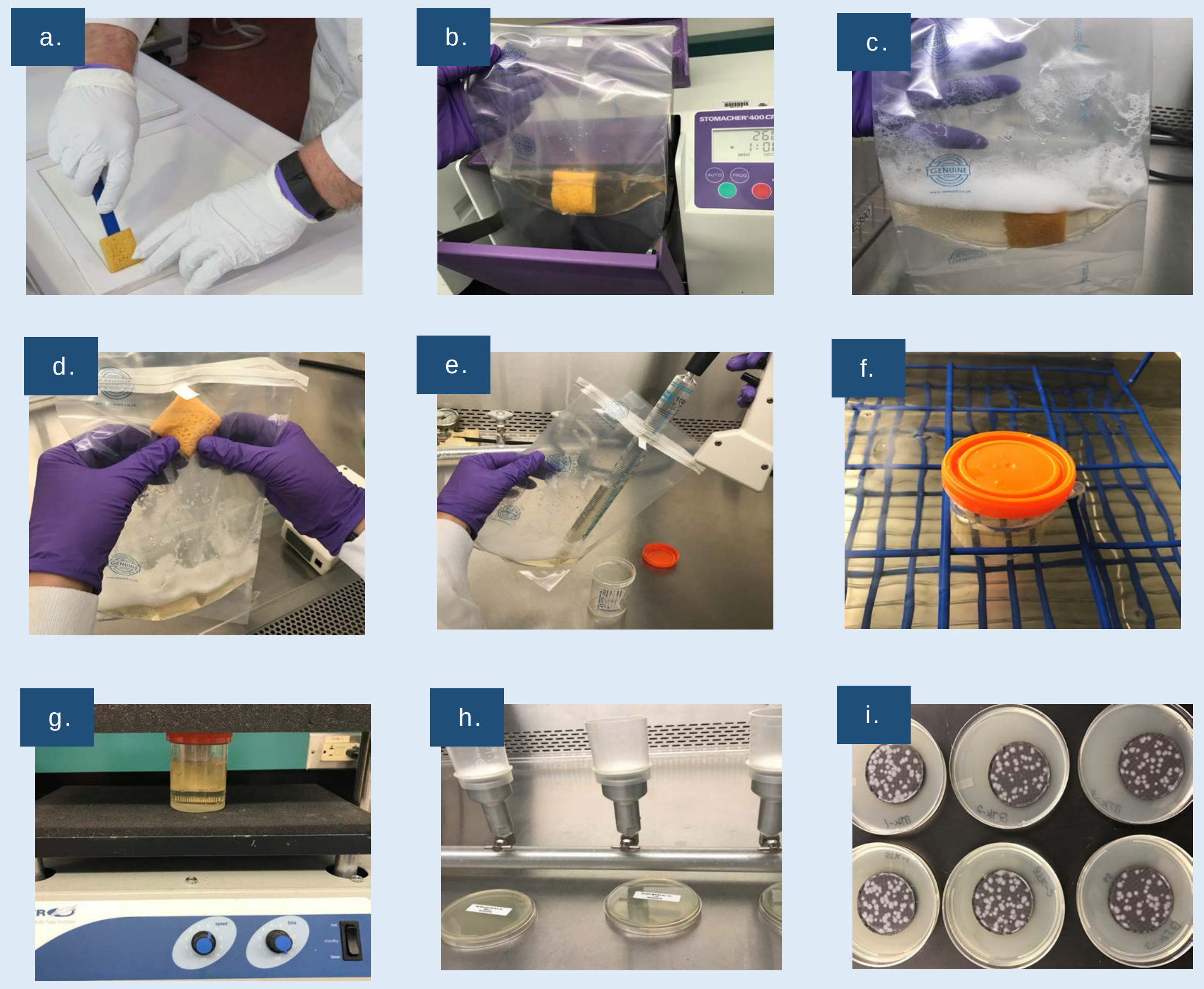


Figure 2 Analytical procedure; (a) sampling, (b – f) extraction, (g – i) analysis.

Efficacy of sample-collection compositing

The current methodology recommended for sampling non-porous smooth surfaces – which utilizes a single implement (sponge 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)

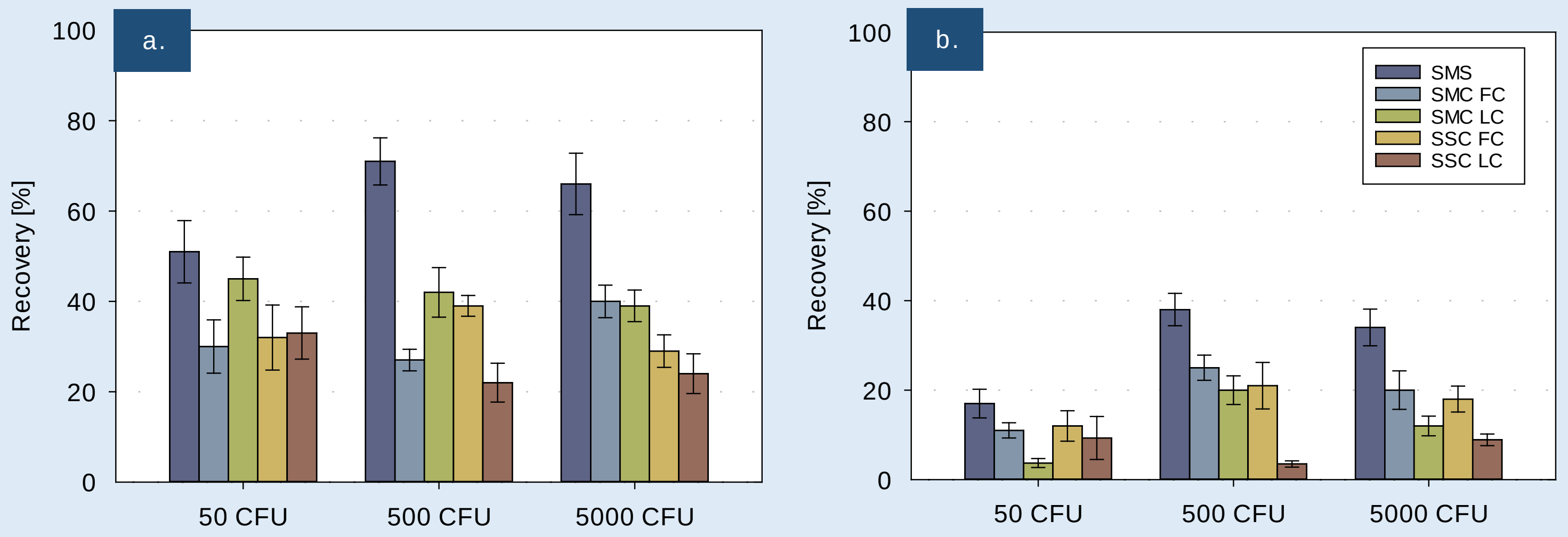


Figure 3 Method-specific recovery (arithmetic mean of % recovery ± 1 SD) of different surface concentrations of target organism from semi-porous (a) and porous material (b)

Both single- and multi-pass 4-point composite (SSC and SMC) methods showed lower %Rec than SMS (Fig. 3), independently of the contamination deposition location (first or last coupon, FC and LC tests in Fig. 3, respectively). The effect of moisture loss from the sponge stick on collection efficiency (LC tests in Fig. 3) was observed in all sample compositing tests, with the average sampling efficacy reduction of 24±13% when compared to the reference method (SMS) results (Fig. 3).

Transfer of contamination during sampling

Contaminant transfer, from first to consecutive coupons, was observed for both composite sampling protocols, but the magnitude of transfer was generally greater when the multi-pass (SMC) protocol was used (Fig. 4a). Noteworthy, the contamination was not detected on coupon R2 (Fig 4b) that was sampled with a side of the sponge stick that was never in contact with the contaminated TC surface during initial stages of SSC sampling..

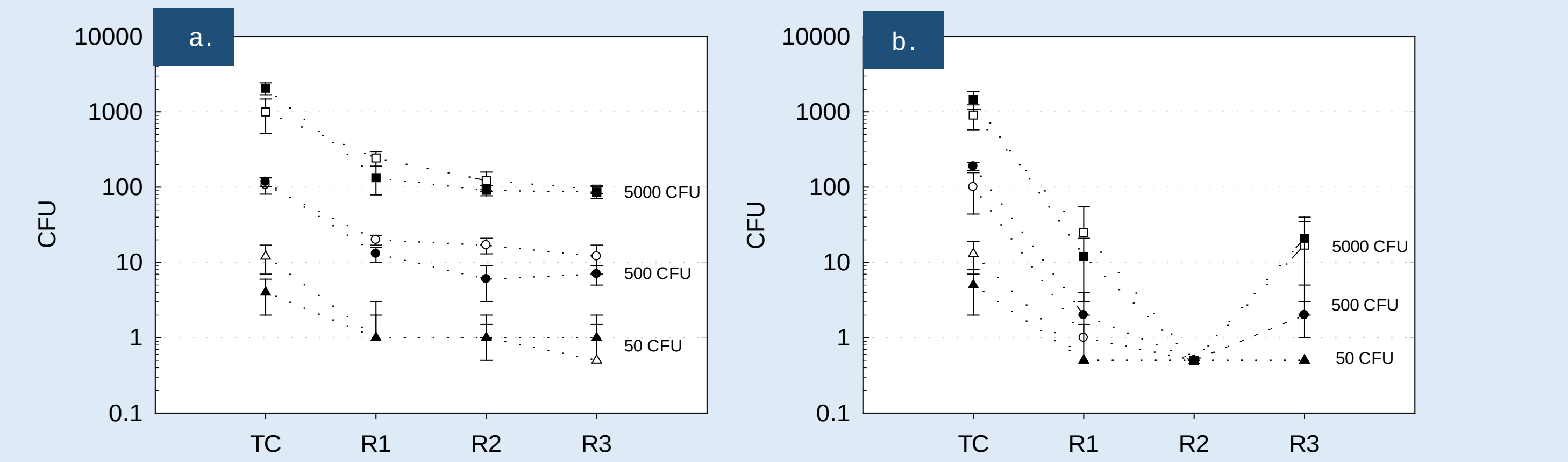


Figure 4 Contamination transfer (arithmetic mean of CFU ± 1 SD) during multi-point sample compositing; (a) SMC sampling, (b) SSC sampling; the contamination was located on the first coupon (TC); coupons R1 through R2 were not contaminated and were resampled using the reference method (SMS) after conclusion of multi-point composite sampling of each set of 4 coupons.

Efficacy of compositing during analysis

Testing of simulated post-sample compositing (or compositing during analysis method, [SPSC]) showed that up to four sponge sticks can be extracted together with no statistically significant difference (ANOVA, $p = 0.21$, $\alpha = 0.05$) in target organism recovery between analytical subsets (Fig. 5).

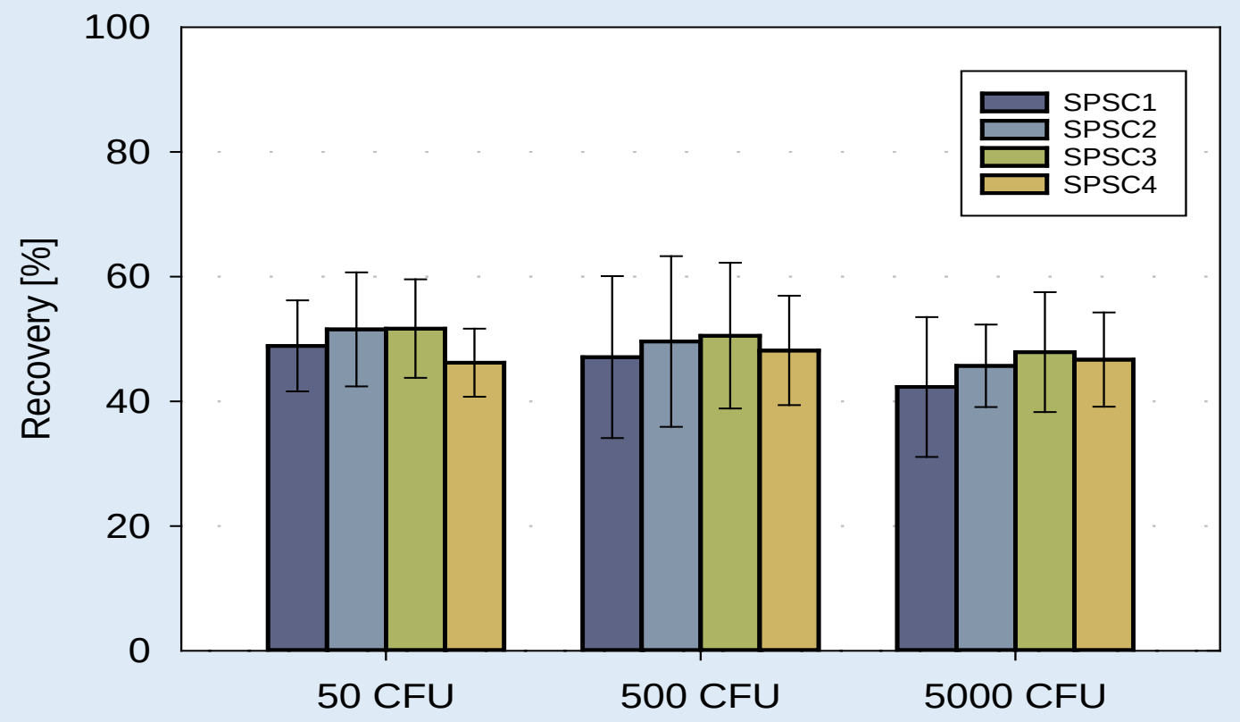


Figure 5 Recovery of different surface concentrations of target organism (arithmetic mean of % recovery ± 1 SD) during composite analysis experiments; samples were collected from semi-porous material (ceramic tile) using SMS method.

Operational time and waste generation

The comparative analysis of laboratory labor (normalized per area sampled) showed that both pre- and post-sample collection compositing 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 ft^2 sampled), followed by SPSC (0.31 lb per ft^2 sampled), and SMS (0.63 lb per ft^2 sampled).

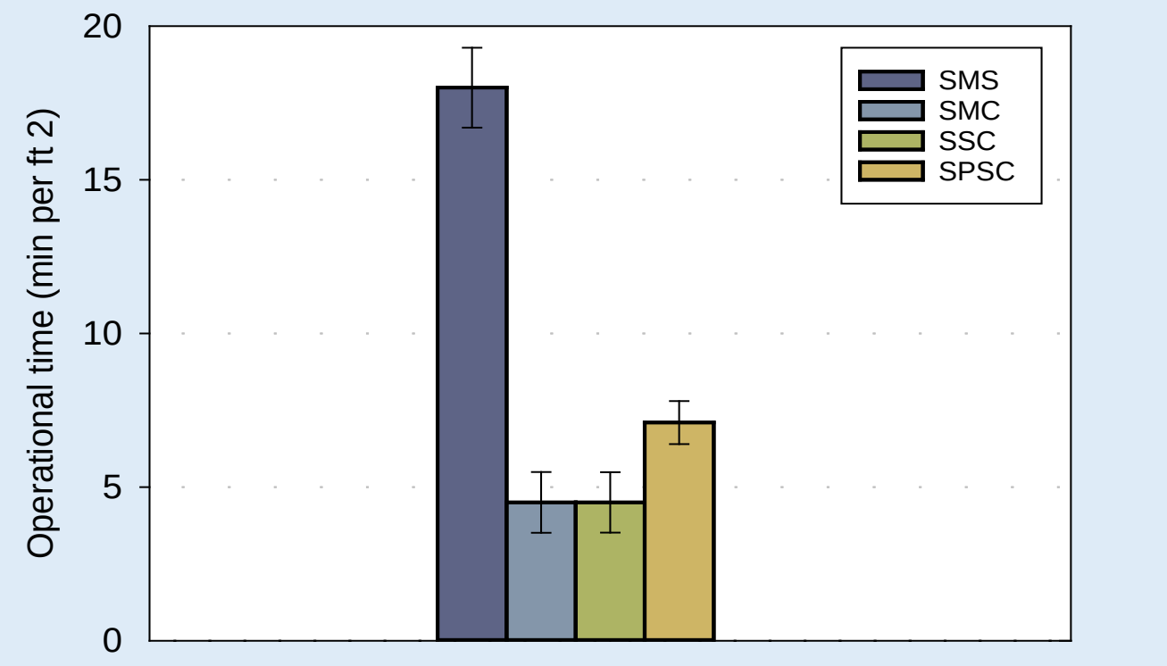


Figure 6 Operational time (arithmetic mean ± 1 SD) for various pre- and post-sample compositing approaches compared to SMS method.

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 SSC and SMC**
- **SSC 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 and time, but at the expense of detection sensitivity**

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