Adaptation of Dry Collection Methods to Quantify Extraction Efficiency of Staphylococcus aureus from Environmental Samples

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Abstract

The Gram-positive bacterium, Staphylococcus aureus can survive in indoor environments in the community, such as schools and homes, contributing to public health concerns related to human exposure and transmission. While convenient methods that do not require refrigeration or surface wetting have been described for identification of environmental S. aureus, these methods currently only provide a positive or negative result. For this project, S. aureus ATCC 43300 was inoculated onto autoclaved Swiffer cloths. Then, S. aureus colonies were extracted from the cloths in 100ml of 1x solution phosphate buffered saline (PBS), the PBS extract was concentrated by vacuum filtration, and colony forming units (CFUs) enumerated on CHROMagar staph agar. S. aureus was successfully enumerated from experimentally-inoculated cloths. The findings from this work demonstrate that S. aureus can be recovered and quantified from dry cloth surface samples. This work also displays that the culture independent method was optimum for extraction efficiency and ease of use. This work highlights the importance of methodological development for S. aureus exposure assessment from indoor community environments.
Goal: Therefore, the goal of this project was to adapt and validate a dry collection method to provide quantification of *S. aureus* from indoor environmental samples comparing culture-based and culture-independent approaches, and then apply this method to environmental surface samples from local schools

Methods

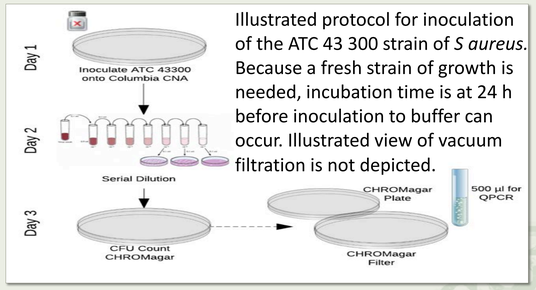
Environmental Sampling (Phase 1)

- Autoclaved Swiffers and sterile swabs sampled from touched surfaces (Figure 3)

Laboratory Methods (Phases 1 & 2)

- ATC 43300 was inoculated onto Columbia CNA Blood Agar to be used in serial dilutions (Figure 1)
- Four samples containing post-Swiffer solution from ATCC 43300 were taken through a filtration process.
- Aliquots from each run taken for DNA extraction
- qPCR performed according using a published protocol (Klotz et al., 2003, p. 4684)
- Gene copies were calculated from CT values based on plasmid standards.

Figure 1. Illustration of inoculation protocol



Illustrated protocol for inoculation of the ATC 43 300 strain of *S aureus*. Because a fresh strain of growth is needed, incubation time is at 24 h before inoculation to buffer can occur. Illustrated view of vacuum filtration is not depicted.

Results

Phase I: Swiffer vs. Swab

- Side-by-side surface (11) samples were collected from four local schools
- CFUs were always recovered from Swiffer samples (Table 1)
- Swabs failed to detect *S. aureus* in 8/11 (73%) of paired samples

Phase 2: Filtration vs. qPCR

- The filtration method produced CFU counts of 31, 56 and TNTC after dilution step (Table 2)
- qPCR method produced results of 304.2982, 30689.21 and 80300.44 (Table 2)

Table 1. Comparison of CPS Swab and CFU Swiffer.

SWIFFER CFUs	SWAB 0	20	40	50
0	0	0	0	0
5	1	0	0	0
7	1	0	0	0
16	1	0	0	0
25	1	0	0	0
33	1	1	0	0
36	1	0	0	0
43	0	0	1	0
47	0	0	0	1
54	2	0	0	0

Figure 2. Filtration Sequence

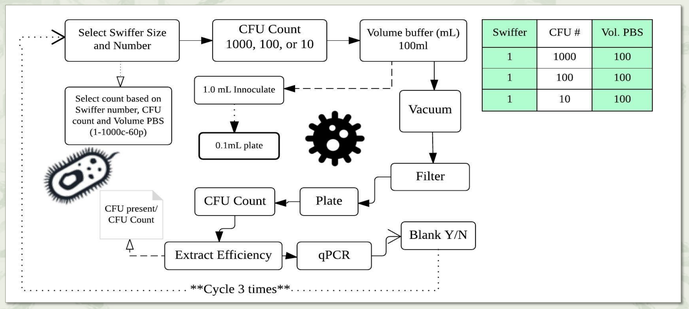


Figure 3. Environmental sampling using Swiffers and on touched and repository surfaces

Figure 4. qPCR Results

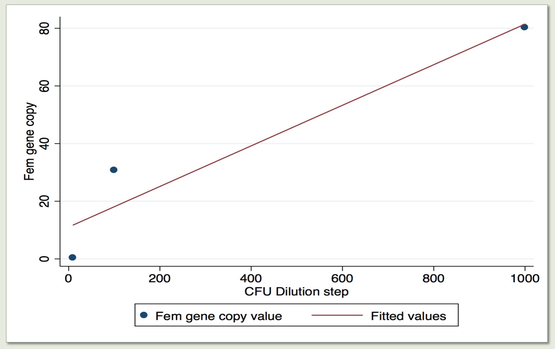


Table 2. Comparison of filtration and qPCR

Fem copy per Swiffer	Filter CFU Quant. 31	56
304	1	0
20689	0	1

Conclusions

- The dry collection method more effective at obtaining *S. aureus* in indoor environments
- The filtration method was resource intensive and challenging
- Filtration method was prone to error due to the volume of buffer used
- The filtration method could be optimized for a lab scarce setting by using smaller buffer volume
- qPCR yielded a quality result that was more feasible and cost efficient

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