Swab methods for environmental SA

Swab type, moistening, and pre-enrichment for *Staphylococcus aureus* on environmental surfaces

Timothy F. Landers

Armando Hoet

Thomas E. Wittum

1 College of Nursing, 1585 Neil Avenue, Columbus, OH 43210

2 College of Veterinary Medicine, 1900 Coffey Road, Columbus, OH 43210

Work was performed at College of Veterinary Medicine, The Ohio State University

*Correspondence:* Timothy Landers

College of Nursing

The Ohio State University

376 Newton Hall

1585 Neil Avenue

Columbus, OH 43210

landers.37@osu.edu

ph (614) 292-0309

RUNNING HEAD: Swab methods for environmental SA
We compared five swabs dry or pre-moistened with or without pre-enrichment to detect surface contamination. Sensitivity varied based on swab type including rayon (71.9% & 75%), cotton (71.2%), polyester (81.3%) and calcium alginate (53.2%). Pre-enrichment improved sensitivity (80% vs. 61.3%) as well as premoistening (83.4% vs. 57.5%). All of the premoistened, pre-enriched swabs were positive.
INTRODUCTION

In the healthcare setting, it is believed that transmission of *Staphylococcus aureus* occurs through transfer from colonized or infected individuals, from the hands of healthcare workers, and through contact with contaminated objects in the environment. (1, 5) SA and methicillin-resistant *Staphylococcus aureus* (MRSA) have been shown to persist for weeks on many items in the healthcare setting including patient care equipment, uniforms, keyboards, cell phones, and ID badges. (6) Monitoring environmental contamination has been recommended as a measure to direct cleaning and decontamination efforts in order to control outbreaks and reduce transmission. (2, 4)

Environmental surveillance is typically performed on surfaces using a sterile swab to recover organisms from surfaces. Commercially available swabs are made of cotton, rayon, polyester (including Dacron®), or calcium alginate material at the end of a plastic, metal, or wooden shaft. Other surface collection methods include the use of wipe sampling devices such as synthetic fabrics or sponges, quantitative cultures using contact plates, and ATP bioluminescence. (3, 9, 10) No standard method for environmental monitoring exists; pre-moistening of the swab tip with a sterile solution is often reported and pre-enrichment of samples is inconsistently reported in studies of environmental contamination. (7)

In order to compare methods of environmental sample collection, we compared five swabs for the detection of *S. aureus* from environmental surfaces, the impact of pre-moistening the swab tip, and the effect of pre-enrichment.

MATERIALS AND METHODS

Eight representative surfaces (keyboard, computer mouse, door knob, mobile phone keypad, faucet handle, light switch, and two flooring samples) were included in the study. Five
swabs were used in random sequence including a commercially available rayon-tipped BBL CultureSwab collection and transport swab (Cat # 220093, BD Diagnostic Systems, Sparks, MD), a cotton-tipped applicator with wooden shaft (Q-Tip, Kendall Healthcare Products, Mansfield, MA), a plastic shaft, polyester-tipped applicator (Part# 36816, Solon, Solon, ME), an aluminum shaft calcium alginate nasopharyngeal applicator (Part #36600, Solon, Solon, ME), and samples of a rayon swab used for the recovery of fastidious organisms (Quest Diagnostics, Pittsburgh, PA). Sterile 0.9% sodium chloride, USP (Baxter Healthcare, Deerfield, IL) was used to premoisten each swab when required.

Each surface was disinfected and then contaminated in a biosafety cabinet by spraying a known solution of *S. aureus* (ATCC 25923) in tripticase soy borth (TSB, BD BBL) at approximately 1.28 × 10^5 CFU/cm². Surfaces were allowed to air dry for 24 hours prior to the surface collection. A 5 x 5cm template was placed in the sampling area to approximate the desired sampling surface. The first series of sampling was done with swabs as supplied by the manufacturer (“dry swab”) and then sampling was repeated using a swab that had been pre-moistened in 0.9% normal sterile saline (“wet swab”).

In order to replicate handling procedures at our facility, all swabs were held at room temperature for 8 hours and then streaked on to MSA (MSA, BD BBL, Sparks, MD). Following inoculation of the first plate, swabs were pre-enriched by placing them in 5ml of TSB (TSB, BD BBL, Sparks, MD) for 12 hours and then re-plated to MSA. Incubation was performed at 37° C and plates were examined for growth at 12, 24, and 48 hours. Fisher’s exact test was used to calculate statistically significant differences by swab type and by pre-enrichment status.

RESULTS
There were a total of 140 samples available for analysis. Rayon-tipped swabs, polyester, plain rayon, and cotton tipped applicators had similar positive rates (71.9%, 71.2%, 81.3%, and 75%, respectively). Calcium alginate nasopharyngeal applicators had the lowest overall recovery (53.2%; p=.02 vs. rayon swab).

Pre-enrichment markedly improved the rate of detection with 64/80 (80%) of pre-enriched samples positive compared to 49/80 (61.3%) of direct plated specimens (p<.01).

Despite the additional time required for processing, pre-enriched swabs were also positive earlier in the test process and qualitatively more growth occurred on pre-enriched specimens.

Premoistening the swab tip also improved recovery with 67/80 (83.4%) of wet swabs positive and 46/80 (57.5%) of dry swabs positive (p<.01). Regardless of the swab type, 100% of premoistened, pre-enriched samples were positive.

**DISCUSSION**

This study highlights the importance of careful technique when performing environmental sampling. Consistent with findings from other authors, we found that sensitivity increased with use of pre-enrichment regardless of the type of swab used for collection. This was observed despite using the same swab for direct plating and pre-enrichment steps which would be expected to reduce bacterial counts in the second step. It is possible that pre-enrichment allows organisms recovered from dry surfaces to enter a growth phase prior to plating on selective media. While premoistening of the swab tip has not been universally adopted, these findings suggest that this should be routine practice. Future studies should examine the impact of recovery of staphylococcus *in vivo* using premoistened swabs or wetting of mucosal surfaces.
One drawback of this study is that it was conducted in a controlled environment in which a single strain was introduced. Since it is unlikely that surface contamination occurs with a single species in clinical settings, these results should be duplicated in an actual clinical environment. The surfaces in this study were contaminated with a much higher concentration of bacteria than is typically observed in the wild and we expect that these results may over-estimate the sensitivity of each method which makes the variability even more striking. Secondly, we did not test differences depending on the type of surface. Moistening of the swab tip may be more beneficial on heavily textured or irregular surfaces as compared to hard, flat surfaces which could have implications for monitoring contamination on surfaces such as mobile computing platforms or ergonomic keyboards. (8, 11) The results may also be influenced by swab pressure, swab angle, duration of contact, and stroke pattern. While we attempted to control for these variables by having a single technician obtain samples in a controlled environment, these factors may also influence swab performance.

In summary, this study demonstrates the importance of specimen collection methods – specifically swab type and premoistening -- and the improvement in sensitivity with pre-enrichment for the recovery of staphylococci from environmental surfaces.
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