*Infection Control & Hospital Epidemiology* (2023), **44**, 834–836
doi:10.1017/ice.2022.101

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## Concise Communication

# Surface area matters: An evaluation of swabs and surface area for environmental surface sampling of healthcare pathogens

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**Abstract**

Flocked and foam swabs were used to sample five healthcare pathogens from three sizes of steel and plastic coupons; 26 $cm^2$, 323 $cm^2$, and 645 $cm^2$. As surface area increased, 1–2 $log_{10}$ decrease in recovered organisms ($P < .05$) was observed. Sampling 26-$cm^2$ yielded the optimal median percent of pathogens recovered.

(Received 3 January 2022; accepted 25 March 2022; electronically published 13 June 2022)

Environmental surfaces are known to contribute to the transmission of healthcare-associated infections (HAIs). No standard sampling protocols are available for environmental surface sampling in healthcare settings.[1] Standardized, efficient sampling methods that include recommendations for optimum surface area to sample would provide confidence in the detection and quantification of surface contamination levels and would assist in investigations of transmission dynamics. We investigated the influence of surface area size and material on the recovery efficiency of flocked and foam swabs when each of 5 bacterial pathogens were sampled from steel and plastic coupons, typical fomite materials observed in the healthcare setting.

## Methods

Stainless steel (T-304 alloy, 24-gauge, Steward Stainless Supply, Suwanee, GA) and plastic (Kydex-T, 0-80 thickness, P1 Haircell texture, Bloomsburg, PA) surfaces were washed, rinsed, and delineated into 3 sizes for comparison of sampling efficiency: 26 $cm^2$, 323 $cm^2$, and 645 $cm^2$. The steel surfaces were sterilized by autoclave at 121°C for 20 minutes, and the plastic coupons were sterilized by ultraviolent radiance ≥40 μW/$cm^2$ for 1 hour.

Suspensions of 5 healthcare bacterial pathogens were prepared. *Staphylococcus aureus* ATCC 43300 (MRSA), vancomycin-resistant *Enterococcus* Van A+256 (VRE), *Acinetobacter baumannii* MLST12 (AB), and carbapenemase-producing KPC+ *Klebsiella pneumoniae* ATCC BAA-1705 (KPC) were incubated overnight on tryptic soy agar with 5% sheep blood. *Clostridioides difficile ATCC 43598* (CD) spores were prepared as described previously.[2] Serial dilutions were prepared for vegetative cells and spores then were adjusted to a final concentration of $10^5$ colony-forming units (CFU)/mL in a body fluid simulant (artificial test soil [ATS], Healthmark Industries, Frasier, MI). Aliquots of 100 μL for the 26-$cm^2$ coupon, 500 μL for the 323-$cm^2$ coupon, and 1,000 μL for the 645-$cm^2$ coupon were placed on each of the 3 surface-area coupons and 2 surface types, resulting in $10^4$–$10^5$ CFU per coupon. The inocula were spread with a cell spreader in a Class II Biological Safety Cabinet (BSC; Nuaire, Plymouth, MN) with airflow on, then were allowed to dry for 1 hour at ambient temperature and humidity in the closed BSC with no airflow before sampling. Sampling was conducted inside the BSC with airflow on, with either a nylon flocked swab (E-swab Copan Diagnostics, Murrieta, CA) or a polyurethane foam swab (Puritan Healthcare, Guilford, ME) premoistened with 100 μL phosphate-buffered saline solution (PBST). Swabs were swiped across the surface in a uniform manner as described previously,[3] then placed in test tubes for 1 hour before processing. Foam swabs were spun in a vortexer and were then sonicated for 3 cycles of 30 seconds each in 5 mL PBST. Flocked swabs were placed in Liquid Amies storage medium provided with the swab (1 mL) and an additional 4 mL PBST then vortexed and sonicated. The eluates were diluted 10-fold in series and cultured at 35°C; MRSA, VRE, and AB on TSA II with 5% Sheep Blood for 18–24 hours, KPC on MacConkey Agar (Becton Dickson, Franklin Lakes, NJ) for 18–24 hours, CD on CCFA-HT (Anaerobe Systems, Morgan Hill, CA) anaerobically for 36–48 hours. The CFUs were counted, and the percent recovered (%R) was determined relative to the inoculum CFU. Statistical significance was set at 0.05, as determined using the Kruskal-Wallis test to compare the surface area sizes in SPSS version 21 statistical software (IBM, Armonk, NY).

## Results

For all organisms evaluated and both swab types, the median %R was significantly greater when sampling from 26-$cm^2$ steel surfaces (median %R, ≤59.7%) than from the 323-$cm^2$ steel surfaces (median %R, ≤9.2%) or 645-$cm^2$ (median %R, ≤4.8%) steel

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**Cite this article:** West RM, *et al.* (2023). Surface area matters: An evaluation of swabs and surface area for environmental surface sampling of healthcare pathogens. *Infection Control & Hospital Epidemiology*, 44: 834–836, https://doi.org/10.1017/ice.2022.101

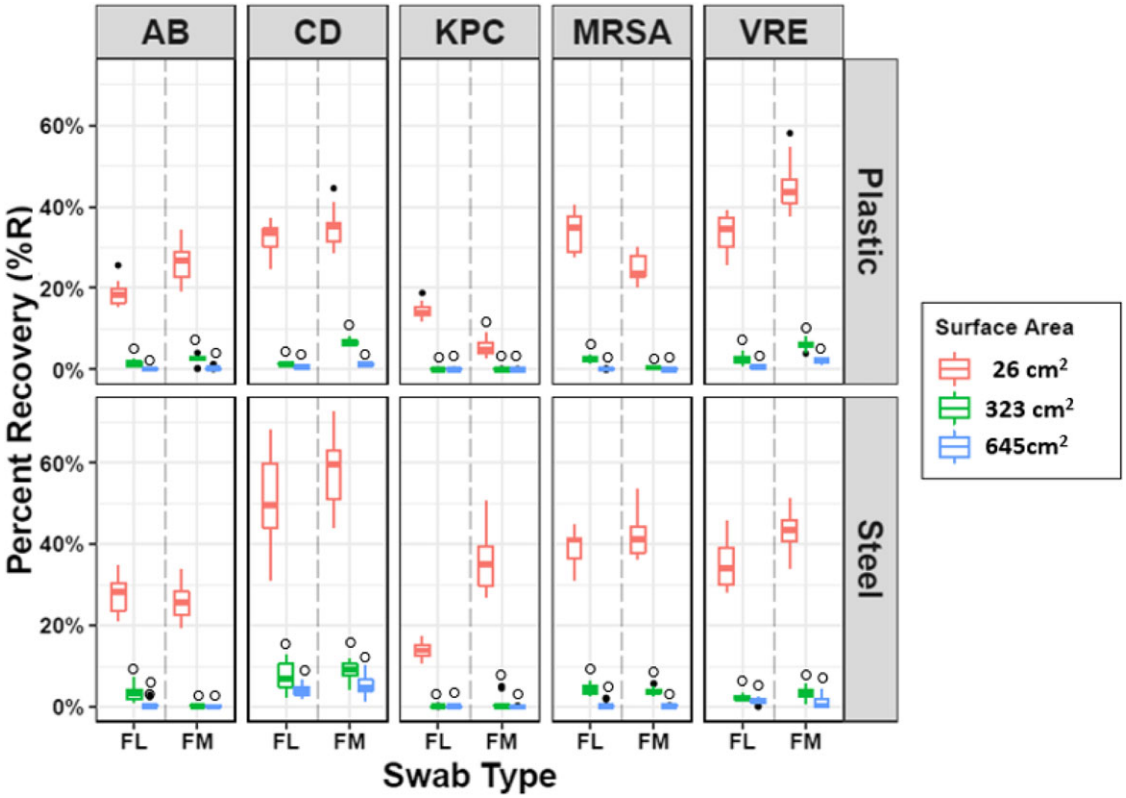


**Fig. 1.** Median percent recovered (%R) of 5 organisms ($10^4$ CFU/coupon) using foam and flocked swabs from 3 surface areas (26 $cm^2$, 323 $cm^2$, and 645 $cm^2$) and 2 surface types (steel and plastic) as suspended in artificial test soil (ATS). Note: Box-and-whisker plot: box; interquartile (IQ) range, line: median, whiskers; maximum and minimum data point, closed circle symbols (•): outliers (likely due to clusters of cells being dispersed during spread-plating), open circle symbols (○): median % R values ≤ 9.2%, red box plot to left (26 $cm^2$), green box plot in the middle (323$cm^2$), blue box plot on the right (645 $cm^2$). Swab types: FM, foam swabs; FL, flock swabs; organisms: AB, *Acinetobacter baumannii*; CD, *Clostridioides difficile*; KPC, *Klebsiella pneumoniae*; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus faecalis* (VRE).

surfaces. Approximately 1 $log_{10}$ fewer organisms (CFU) were recovered from 323-$cm^2$ coupons than from 26-$cm^2$ coupons, and 1–2 $log_{10}$ fewer from 645-$cm^2$ coupons than 26-$cm^2$ (a decrease from 25.0% to 2.5% represents 1 $log_{10}$ reduction) (Fig. 1 and Supplementary Table S1 online). The highest median %R was observed in CD sampled using either foam or flocked swabs from 26-$cm^2$ steel coupons. In contrast, the lowest median %R was observed when KPC was sampled using foam swabs from plastic coupons (Supplementary Table S1 online).

The median %R varied with each organism, as seen in Figure 1, with the %R from 26 $cm^2$ ranging from 14.0% for KPC to 49.6% for CD using the flocked swab and from 4.9% for KPC to 59.7% for CD when using the foam swab. When VRE was sampled from 26-$cm^2$ and 645-$cm^2$ steel surfaces with foam swabs, 2-$log_{10}$ decreases in recovery were observed: 43.5% (SD, 4.4%) for the 26-$cm^2$ steel coupons) and 0.4% (SD, 1.6%) for the 645-$cm^2$ steel coupons.

For all organisms sampled from either surface material, as surface area increased from 26 $cm^2$ to 323 $cm^2$, at least a 1-$log_{10}$ decrease in recovered organisms was detected, and in some cases, a 2-$log_{10}$ reduction was detected (Fig. 1 and Supplementary Table S1 online).

## Discussion

In this study, the %R of the organisms evaluated using flocked and foam swabs decreased significantly with increasing surface area sampled, suggesting that it is best to limit the swab sampling areas to ≤26 $cm^2$. Similar decreases in recovery over larger surface areas have been observed when swabs were used to sample norovirus from steel surfaces.[4] The organisms are most likely absorbed by the swab when it is still moist, then the swab loses moisture as it continues to move across the larger surface areas. As the swab dries, the organisms are more likely to adhere to the surface than the swab, and the organisms are redistributed back onto the subsequent surface areas. Redistribution of *Bacillus atrophaeus* spores onto subsequent surfaces was demonstrated by Tufts et al[5] when using a cellulose sponge sampler. The variability in %R between organisms may be attributed to organism-specific properties that can influence adherence to materials, and to persistence, as discussed in Rose et al.[6] In other studies, researchers have noted that various properties can influence cell adherence to surfaces: hydrophobicity, the charge of the cells, extracellular polysaccharide, pili or flagella, and the presence of organic material, which simulates body fluids encountered in the hospital setting.[7,8] Previous research demonstrated that different sampling devices released organisms into their elution liquids (when processing in the laboratory) to different degrees, suggesting that the physical and chemical properties of the sampling device can influence the %R.[9] The differences in physical properties of the sampling tools (e.g., surface area, hydrophobicity) may explain the differences in %R. Additional factors that may affect recovery efficiency include ambient room temperature and humidity.[10] Further work is needed to address detection

https://doi.org/10.1017/ice.2022.101 Published online by Cambridge University Press

by molecular methods, which may prove helpful when detecting viruses and bacteria not typically detected by culture. These data illustrate the need to limit swab sampling areas to 26 $cm^2$ when sampling for bacterial pathogens in healthcare settings.

**Supplementary material.** To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2022.101

**Acknowledgments.**

**Financial support.** No financial support was provided relevant to this article.

**Conflicts of interest.** All authors report no conflicts of interest relevant to this article.

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