

Detecting *Clostridium difficile* Spores from Inanimate Surfaces of the Hospital Environment: Which Method Is Best?

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The recovery of *Clostridium difficile* spores from hospital surfaces was assessed using rayon swabs, flocked swabs, and contact plates. The contact plate method was less laborious, achieved higher recovery percentages, and detected spores at lower inocula than swabs. Rayon swabs were the least efficient method. However, further studies are required in health care settings.

Clostridium difficile is a spore-forming anaerobic nosocomial pathogen associated with mild to life-threatening diarrhea or colitis (1, 2). *C. difficile* colonization increases with the length of hospital stay following environmental exposure to spores or contact with an infected person (3). Contamination and survival of *C. difficile* spores on hospital inanimate surfaces have been reported (4, 5) and shown to be associated with cross-transmission (6–8).

Inactivation and eradication of *Clostridium difficile* spores are a challenge, and several relatively new techniques have been investigated, such as hydrogen peroxide vapor, UV radiation, or gaseous plasma systems (5, 6, 9, 10). Validation of such techniques should use optimal recovery methods to better quantify bacterial spore killing or eradication.

A variety of methods have been used to detect *C. difficile* from the hospital environment with variable results (11, 12). Here we report an evaluation of different methods to detect and recover *C. difficile* spores from materials commonly found in the hospital environment.

(Preliminary data arising from this study were presented as a poster at the European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain, May 2014.)

One *C. difficile* reference strain, ATCC 700057 (Cruinn Diagnostics, Ireland), and one uncharacterized clinical isolate (Diagnostic Laboratory, Beaumont Hospital, Dublin, Ireland) were included in the study. A modification of the *C. difficile* spore preparation protocol described by Chilton et al. was used (13). Briefly, *C. difficile* was inoculated into prerduced brain heart infusion supplemented with yeast extract and L-cysteine, incubated anaerobically at 37°C for 48 h, and spread onto 10 Columbia blood agar plates (Oxoid, United Kingdom), which were incubated anaerobically at 37°C for 10 days (14). Growth was collected from the plates using a cell scraper and suspended in 1 ml phosphate-buffered saline (PBS)–ethanol (50% [vol/vol]). The suspensions were incubated at room temperature for 1 h with periodic mixing. Spore suspensions were centrifuged for 10 min at 16,000 × g, and the pellets were resuspended in 1 ml of PBS. Spore numbers and purity were determined by microscopy using the Schaeffer and Fulton spore stain kit (Sigma-Aldrich, Ireland). Suspensions were adjusted to a concentration of approximately 10⁵ CFU/ml (one colony is equivalent to one spore), and serial 1:10 dilutions were prepared in PBS.

C. difficile spore suspensions (50 μl), ranging from 10⁵ to 10 CFU/ml, were added in duplicate to 25-cm² sections of polyure-

thane mattress fabric (Meditec Medical, Ireland), polypropylene (GoodFellow Cambridge, Ltd., United Kingdom) and stainless steel, all decontaminated as described previously (15). Spore suspensions applied to the surfaces were air dried over 2 h. The surfaces were sampled using premoistened rayon swabs and nylon flocked swabs (Copan, Italy) and placed in 3 ml PBS. Serial dilutions of the swab suspensions were inoculated onto preprepared *C. difficile* selective plates—*C. difficile* agar base CM0601 plus *C. difficile* supplement SR0096 (250 mg/liter D-cycloserine, 8 mg/liter cefoxitin) and 7% (vol/vol) defibrinated horse blood (SR0050)—provided by Oxoid for enumeration of CFU/ml. Sterile contact plates (VWR) were poured in the laboratory with *C. difficile* selective agar and applied to each section for 20 s, ensuring firm contact with the surface. Subcultured plates from both swabs and contact plates were incubated overnight anaerobically at 37°C. Colony enumeration was performed the following day. The limit of detection (LOD) was defined as the lowest concentration of spores applied that was detected by a specific method. All methods were assessed independently targeting the entire section, and their abilities to detect and recover *C. difficile* spores were compared. Statistical analysis was carried out using GraphPad Prism 5.00 software. The means of three independent experiments of the percentage of recovery for the different methods were analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple-comparison test.

In 1983 one of the first reports comparing methods for the recovery of *C. difficile* spores from an environmental glass surface showed that of swabs, adhesive paddles, and contact plates, contact plates were by far the most efficient method, detecting spores at low levels, being simpler to use and relatively rapid (16). Since then, various methods, including swabs, contact plates, gloves, and sponges, have been used for research and outbreak investigations with variable results. Here, we demonstrate that contact

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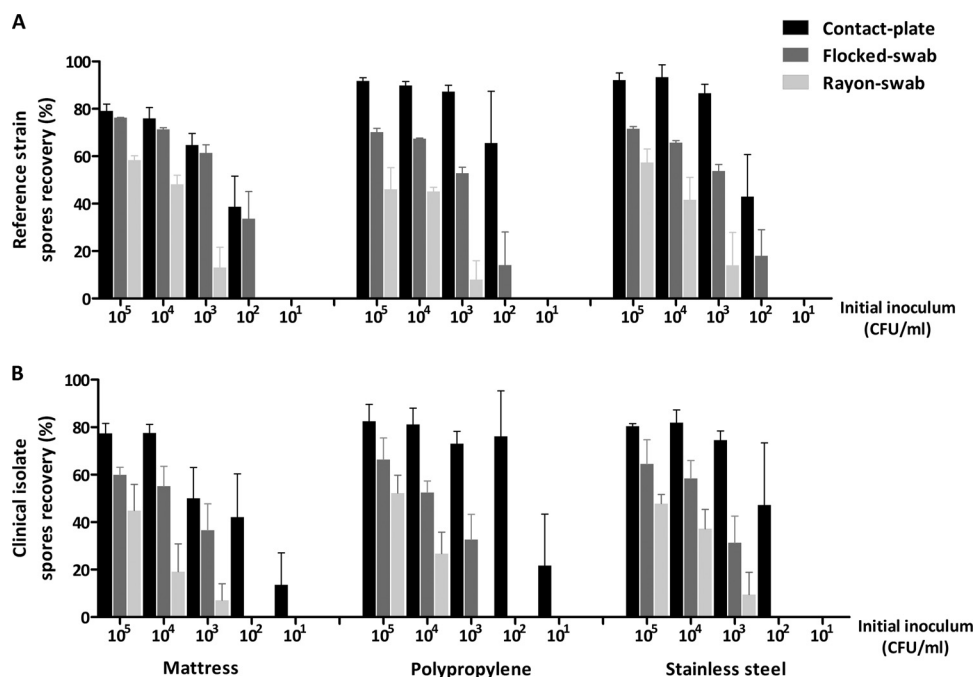


FIG 1 Percentage recovery of *Clostridium difficile* spores of the reference strain (A) and clinical isolate (B) from three environmental surfaces using contact plates, flocked swabs, and rayon swabs. Error bars represent the standard errors of the means (SEM) from at least three independent experiments ($n \geq 3$).

plates achieved the highest recovery of *C. difficile* spores (14% to 92%) from various surfaces, including the mattress material, polypropylene, and stainless steel, confirming and extending the results from Buggy et al. (16) for recovery from glass. Recovery was also efficient for flocked swabs (14% to 76%), and the least efficient method was the rayon swab (7% to 58%) (Fig. 1A and B). The recovery percentage was directly proportional to the size of the initial inoculum—i.e., an inoculum of 10^5 CFU/ml allowed contact plates to recover as much as 92% and as little as 14% for a 10-CFU/ml inoculum. Contact plates were also most sensitive at detecting spores at a minimum inoculum of 10 CFU/ml, while the LOD was 10^2 CFU/ml for flocked swabs and 10^3 CFU/ml for rayon swabs, but this was only true for the clinical isolate. Statistically, contact plates were better than flocked swabs on polypropylene for the clinical isolate ($P < 0.05$) (Table 1) and persistently superior to rayon swabs ($P < 0.05$ to $P < 0.001$) (Table 1), while there

was no significant difference between flocked and rayon swabs. Although statistical analysis did not reveal any significant difference in the recoveries between all surfaces studied, the recoveries from the mattress material were slightly reduced compared to those from the other two surfaces. Furthermore, no statistical difference was seen between the two isolates.

C. difficile spores can be shed to the environment by both asymptomatic and symptomatic patients and may survive for up to 5 months on inanimate surfaces (17). They resist the bactericidal effects of most hospital disinfectants and most other decontamination techniques (18). Therefore, interventions should be monitored in rooms currently or previously occupied by *C. difficile* carriers. The reporting of improved methods in terms of speed and sensitivity, such as the contact plate and flocked swabs reported here, gives a more accurate estimate of the *C. difficile* burden compared to previously reported methods. Given that *C. difficile* is the most prominent endospore in today's health care setting and presents a significant infection risk to vulnerable patients, there is a growing need to accurately quantify *C. difficile* spore contamination in the health care environment. This would be for the purpose of assessing new methods of decontamination and correlating levels of environmental contamination with the risk of infection and the occurrence of outbreaks. Nevertheless, improving accepted recovery methods using materials that represent those found in the hospital setting are a necessary prerequisite.

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TABLE 1 Summary of the results of statistical analysis carried out using one-way ANOVA and Tukey's multiple-comparison test

| Methods compared | P value from statistical analysis of ^a : | | | | |
|-------------------------------|---|----------|----------|---------------|-----------------|
| | Reference | Clinical | Mattress | Polypropylene | Stainless steel |
| Contact plate vs flocked swab | NS | <0.05 | NS | <0.05 | NS |
| Contact plate vs rayon swab | <0.01 | <0.001 | <0.05 | <0.01 | <0.05 |
| Flocked swab vs rayon swab | NS | NS | NS | NS | NS |

^a The results were obtained by statistical analysis of the mean percentages of recovery from three different methods tested on three different surfaces with two isolates of *C. difficile*. NS, not significant.

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