Monitoring the Effectiveness of Hospital Cleaning Practices by Use of an Adenosine Triphosphate Bioluminescence Assay •

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ORIGINAL ARTICLE

Monitoring the Effectiveness of Hospital Cleaning Practices by Use of an Adenosine Triphosphate Bioluminescence Assay

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OBJECTIVE. To evaluate the usefulness of an adenosine triphosphate (ATP) bioluminescence assay for assessing the efficacy of daily hospital cleaning practices.

DESIGN. A 2-phase prospective intervention study.

SETTING. A university-affiliated community teaching hospital.

METHODS. During phase I of our study, 5 high-touch surfaces in 20 patient rooms were sampled before and after daily cleaning. Moistened swabs were used to sample these surfaces and were then plated onto routine and selective media, and aerobic colony counts were determined after 48 hours of incubation. Specialized ATP swabs were used to sample the same high-touch surfaces in the 20 patient rooms and were then placed in luminometers, and the amount of ATP present was expressed as relative light units. During phase II of our study, after in-service housekeeper educational sessions were given, the housekeepers were told in advance when ATP readings would be taken before and after cleaning.

RESULTS. During phase I, the colony counts revealed that the 5 high-touch surfaces were often not cleaned adequately. After cleaning, 24 (24%) of the 100 surface samples were still contaminated with methicillin-resistant *Staphylococcus aureus,* and 16 (16%) of the 100 surface samples still yielded vancomycin-resistant enterococci. ATP readings (expressed as relative light units) revealed that only bathroom grab bars and toilet seats were significantly cleaner after daily cleaning than before. During phase II, a total of 1,013 ATP readings were obtained before and after daily cleaning in 105 rooms. The median relative light unit was significantly lower (ie, surfaces were cleaner) after cleaning than before cleaning for all 5 high-touch surfaces.

CONCLUSIONS. Suboptimal cleaning practices were documented by determining aerobic colony counts and by use of an ATP bioluminescence assay. ATP readings provided quantitative evidence of improved cleanliness of high-touch surfaces after the implementation of an intervention program.

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Transmission of healthcare-associated pathogens most frequently occurs via the transiently contaminated hands of healthcare workers.[1] However, environmental contamination also contributes to the spread of healthcare-associated pathogens.[2-9] As a result, hospitals need to ensure that environmental cleaning and disinfection are integral parts of their infection control programs.[10-12]

However, routine housekeeping practices are often suboptimal,[3,13-17] and increased attention should be paid to the effectiveness of cleaning protocols. Accordingly, the Hospital of Saint Raphael formed a multidisciplinary committee to revise and update the hospital's policies. After formal acceptance of the revised and updated policies by the infection control program and environmental services, a decision was made to monitor the effectiveness of cleaning procedures.

Methods for monitoring the effectiveness of cleaning procedures include visual assessment of surfaces, application of fluorescent dye to surfaces with subsequent assessment of residual dye after cleaning, determination of aerobic colony counts, and detection of adenosine triphosphate (ATP) on surfaces.[13,15,18,19] Detection of ATP—which is present in all types of organic material (including bacteria, food, and human secretions and excretions)—on environmental surfaces has been used for years in the food and beverage industries to assess the adequacy of cleaning procedures.[19,20] Few investigators have evaluated ATP bioluminescence methods for

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monitoring cleanliness in hospitals.[13,19,21] Therefore, we conducted a 2-phase prospective intervention study of the usefulness of an ATP bioluminescence assay to assess the adequacy of routine hospital cleaning procedures.

METHODS

Phase I

Phase I was designed to assess the thoroughness of daily cleaning procedures by determining aerobic colony counts and by use of an ATP bioluminescence assay and to compare the results of the 2 methods. We felt that expressing surface contamination as an aerobic colony count as well as an ATP reading would make it easier for hospital personnel to comprehend the results. During the first phase of the study, the following 5 high-touch surfaces in patient rooms were selected for sampling before and after daily cleaning by housekeepers: bedside rails, overbed tables, television remote controls, toilet seats, and bathroom grab bars in patient bathrooms. Surfaces were sampled for culture shortly before daily cleaning. Samples were obtained after the housekeeper had exited the room and after disinfectant had been allowed to dry for at least 10 minutes. Because of the nonuniform surfaces sampled, we were unable to sample a standardized area on each surface. Sampling included approximately one-eighth to one-fourth of the surface of an overbed table, the entire television remote control, 12 inches of the grab bars and top surface of the upper bedside rails, and one-half of the toilet seat. Surfaces were sampled by use of moistened swabs, which were used to inoculate blood agar plates, chromogenic methicillin-resistant *Staphylococcus aureus* (MRSA) selective agar plates (CHROMagar MRSA; BD Diagnostics), and *Campylobacter* agar plates and then placed in broth enrichment. No neutralizers were incorporated into the agar or broth used for culture. Broth cultures were inoculated onto the same agar plates after 24 hours of incubation. Total aerobic colony counts were determined after 48 hours of incubation. Mauve colonies growing on chromogenic MRSA selective agar were classified as MRSA after use of a confirmatory coagulase test. Colonies growing on *Campylobacter* agar that were morphologically consistent with enterococci, that tested positive for pyrrolidonyl arylamidase, and that grew on brain-heart infusion agar plates containing 6 μg/mL vancomycin were considered to be vancomycin-resistant *Enterococcus* (VRE).

An ATP bioluminescence assay (3M Clean-Trace ATP System; 3M) was used to assess the level of cleanliness of surfaces.[20] This assay includes specialized swabs for sampling surfaces, ATP bioluminescence reaction tubes, hand-held programmable luminometers for detecting and recording the amount of ATP present on swabs, and a customized database that is used to store and analyze results. At the same time that moistened swabs were used to sample the 5 high-touch surfaces for culture, ATP swabs were used to sample the surfaces immediately adjacent to the areas sampled for culture. These specialized swabs were placed into ATP bioluminescence reaction tubes and agitated for at least 5 seconds. During this time, the following reaction occurred:

$$\text{luciferase} + \text{D-luciferin} + O_2 + \text{ATP} \Rightarrow \text{luciferase} + \text{oxyluciferin} + CO_2 + \text{AMP} + PP_i + \text{light},$$

where AMP is adenosine monophosphate and PP_i is inorganic pyrophosphate.

The amount of light (ie, bioluminescence) generated is proportional to the amount of organic material present on the swabs; organic material contains ATP, which emits light when combined with the compounds in the ATP bioluminescence assay. After the reaction tubes containing the swabs were agitated, the reaction tubes were inserted into a luminometer, which provides a digital readout of the amount of light generated by the luciferase reaction, expressed as relative light units (RLUs). Well-cleaned surfaces with very little organic material present yielded less than 250–300 RLUs, whereas poorly cleaned surfaces with a lot of organic material present yielded more than 1,000 RLUs. The ATP readings obtained from the 5 high-touch surfaces before and after daily room cleaning were uploaded from the luminometer into the customized database for further analysis. The samples were obtained by a member of the infection control program from a convenience sample of 20 patient rooms to determine aerobic colony counts and ATP readings. Housekeepers were not notified that monitoring of cleaning practices was being performed.

Phase II

The major goal of phase II of our study was to establish with greater certainty the range of ATP readings to be expected on high-touch surfaces in patient rooms before and after daily cleaning. A secondary goal was to determine whether alerting housekeepers that cleaning procedures were being monitored would result in improved cleaning practices, as reflected in the ATP readings. At the beginning of phase II, in-service educational sessions regarding the role contaminated environmental surfaces play in the transmission of pathogens, the importance of daily cleaning, and the results of phase I were presented to housekeepers by an infection control practitioner. During the second phase of our study, 2 environmental services managers were instructed on how to use the ATP swabs and luminometers. Before obtaining samples of the 5 high-touch surfaces in a patient room, the managers notified housekeepers that they would be obtaining ATP readings of the 5 high-touch surfaces before and after cleaning. Housekeepers were aware of which surfaces were being monitored. ATP readings were obtained in patient rooms located on all medical and surgical wards. The wards where the sampling was performed were randomized by use of SPSS software, version 10.1.0 (SPSS). This was done to ensure that the sam-

ples were obtained in rooms occupied by different types of patients and that the rooms were cleaned by a variety of housekeepers. The individual patient rooms to be sampled were not randomized.

Hospital Cleaning Methods

Daily cleaning of the patient rooms included in our study was performed with the use of a detergent disinfectant containing 660 ppm of active quaternary ammonium (Virex II 256; JohnsonDiversey). Wipes submerged in buckets containing the disinfectant were used to clean surfaces. Rooms disinfected with 10% household bleach were not included, because high concentrations of bleach can quench the ATP bioluminescence reaction.

Statistical Analysis

The data collected from all of the samples were transferred to SPSS software, version 10.1.0 (SPSS), for statistical analysis. The median aerobic colony count and the median RLU were determined for each of the 5 high-touch surfaces before and after daily cleaning. Paired data were analyzed by use of the Wilcoxon signed ranks test. When comparing ATP readings after daily cleaning during phases I and II, the data were analyzed by use of the Mann-Whitney *U* test.

RESULTS

Phase I

Colony counts obtained before and after cleaning in the 20 patient rooms varied considerably for all 5 high-touch surfaces (Table 1). The proportions of surfaces with a colony count after cleaning that was lower than before cleaning were as follows: 12 (60%) of 20 bedside rails, 6 (30%) of 20 overbed tables, 5 (25%) of 20 television remote controls, 11 (55%) of 20 bathroom grab bars, and 14 (70%) of 20 toilet seats. The median colony counts obtained after cleaning were significantly lower than those obtained before cleaning for bathroom grab bars ($P = .02$) and toilet seats ($P = .03$) only (Table 1).

The proportions of samples for culture that were positive for MRSA before cleaning were as follows: 12 (60%) of 20 bedside rails, 9 (45%) of 20 overbed tables, 9 (45%) of 20 television remote controls, 4 (20%) of 20 bathroom grab bars, and 6 (30%) of 20 toilet seats. The proportions of samples for culture that were positive for MRSA after cleaning were as follows: 9 (45%) of 20 bedside rails, 8 (40%) of 20 overbed tables, 4 (20%) of 20 television remote controls, 3 (15%) of 20 bathroom grab bars, and none (0%) of 20 toilet seats. Of the 100 surface samples tested by culture, 40 (40%) were positive for MRSA before cleaning, and 24 (24%) were positive for MRSA after cleaning. For surface samples that were positive for MRSA by direct plating, the median colony count on culture was less than 5 for all surfaces, except overbed tables after cleaning (median colony count on culture, 24) and television remote controls after cleaning (median colony count on culture, 15).

The proportions of samples for culture that were positive for VRE before cleaning were as follows: 6 (30%) of 20 bedside rails, 8 (40%) of 20 overbed tables, 2 (10%) of 20 television remote controls, 3 (15%) of 20 bathroom grab bars, and 5 (25%) of 20 toilet seats. The proportions of samples for culture that were positive for VRE after cleaning were as follows: 3 (15%) of 20 bedside rails, 3 (15%) of 20 overbed tables, 4 (20%) of 20 television remote controls, 2 (10%) of 20 bathroom grab bars, and 4 (20%) of 20 toilet seats. Of the 100 surface samples tested by culture, 24 (24%) were positive for VRE before cleaning, and 16 (16%) were positive for VRE after cleaning. For surface samples that were positive for VRE by direct plating, the median colony count on culture was less than 10 for all surfaces, except bathroom grab bars after cleaning (median colony count on culture, 100) and toilet seats before cleaning (median colony count on culture, 65).

ATP readings (expressed as RLUs) that were obtained before and after cleaning in 20 patient rooms also varied considerably for the 5 high-touch surfaces (Table 1). The proportions of surface samples with a median RLU value that was lower after cleaning than before cleaning were as follows: 7 (35%) of 20 bedside rails, 10 (50%) of 20 overbed tables, 12 (60%) of 20 television remotes controls, 16 (80%) of 20 bathroom grab bars, and 16 (80%) of 20 toilet seats. The median RLU values obtained after cleaning were statistically significantly lower than those obtained before cleaning only for bathroom grab bars ($P = .03$) and toilet seats ($P = .01$) (Table 1).

The aerobic colony counts obtained before and after clean-

TABLE 1. Phase I Data on Samples Obtained From 5 High-Touch Surfaces in 20 Patient Rooms, Before and After Daily Cleaning, at the Hospital of Saint Raphael

Unit of measure, time of sampling	Bedside rails	*P*	Overbed tables	*P*	Television remote controls	*P*	Bathroom grab bars	*P*	Toilet seats	*P*
Median ACC on culture (range)		.07		.20		.55		.02		.03
Before cleaning	43 (1 to >100)		21 (2 to >100)		20 (0 to >100)		9 (0 to >100)		14.5 (2 to >100)	
After cleaning	19 (4 to >100)		57.5 (1 to >100)		15 (0 to >100)		2 (0 to >100)		1 (0 to >100)	
Median RLU values (range)		.17		.60		.23		.03		.01
Before cleaning	275 (73–3,070)		212 (15–13,413)		324 (54–7,993)		431 (40–1,987)		293 (64–4,744)	
After cleaning	614 (32–3,254)		201 (9–2,658)		187 (50–2,296)		182 (33–2,338)		82 (12–6,488)	

NOTE. ACC, aerobic colony count; RLU, relative light unit.

ing were combined and compared with the RLU values obtained both before and after cleaning. There was a low, albeit statistically significant, correlation between colony counts and RLU values for each of the 5 high-touch surfaces, with correlation coefficients ranging from 0.356 to 0.649 (Table 2).

Phase II

A total of 1,013 ATP readings were obtained from the 5 high-touch surfaces before and after daily cleaning of 105 patient rooms on 16 wards. The RLU values obtained from the samples of the high-touch surfaces before and after cleaning are shown in Table 3. The proportions of surface samples with a median RLU value that was lower after cleaning than it was before cleaning were as follows: 76 (74%) of 103 bed rails, 85 (83%) of 102 overbed tables, 72 (71%) of 101 television remotes controls, 72 (73%) of 99 bathroom grab bars, and 69 (70%) of 98 toilet seats. The median RLU values obtained after cleaning were statistically significantly lower than those obtained before cleaning for all 5 high-touch surfaces (Table 3).

A comparison of the RLU values obtained after cleaning during phase I (when housekeepers were unaware that ATP readings were being taken) with those obtained after cleaning during phase II (when housekeepers had already gone to in-service educational sessions and were told in advance that ATP readings would be taken) revealed that the median RLU values were significantly lower during phase II than during phase I, except for toilet seats, which revealed low RLU values during phase I (Figure).

TABLE 2. Correlation Between Aerobic Colony Counts and Relative Light Unit Values for Samples Obtained From 5 High-Touch Surfaces in 20 Patient Rooms at the Hospital of Saint Raphael

High-touch surface sample	Spearman rank correlation coefficient	*P*
Bedside rail	0.356	.024
Overbed table	0.428	.006
Television remote control	0.401	.011
Bathroom grab bar	0.385	.018
Toilet seat	0.649	<.001

NOTE. The aerobic colony counts obtained both before and after cleaning were compared with the relative light unit values obtained both before and after cleaning.

DISCUSSION

We used both aerobic colony counts and the detection of ATP to monitor the effectiveness of daily cleaning of 5 high-touch surfaces in patient rooms, and we established that housekeepers were not adhering to a set of newly implemented cleaning policies. On the basis of these findings, new educational programs were developed and presented to housekeepers, and discussions were held with environmental services managers regarding the deficiencies identified. Subsequently, housekeepers were notified in advance when the patient rooms to be cleaned would be checked after cleaning. This combination of measures resulted in significant improvement in the cleanliness of all 5 high-touch surfaces, as reflected in the reduced levels of ATP observed on the surface samples after daily cleaning.

In many hospitals, it is likely that there has been little assessment of the adequacy of routine housekeeping practices. Recent studies have documented that cleaning of patient care areas is often suboptimal and that surfaces may remain contaminated with pathogens after routine cleaning.[3,13-17] In some hospitals, visual inspection of cleaned surfaces has been assumed to be adequate. However, surfaces that meet visual criteria for cleanliness often remain contaminated with microorganisms or other organic material.[19,21-23] As a result, more quantitative methods are warranted to adequately assess the effectiveness of cleaning practices.[19]

Our phase I finding that, after the cleaning of some surfaces, the colony counts and ATP readings were not significantly lower than those obtained before cleaning is consistent with other studies demonstrating that 45%–50% of surfaces that should be cleaned are suboptimally cleaned.[3,15] The occurrence of colony counts and ATP readings that were higher after cleaning than before cleaning has also been reported elsewhere.[19] When colony counts and ATP readings in the present study documented that surfaces were not always cleaned appropriately, discussions with housekeepers and environmental services managers identified several obstacles to appropriate cleaning of surfaces that were successfully overcome.

Comparing the aerobic colony counts observed in our study with those reported in earlier studies is problematic because the sampling methods that we used were different from those used by some other investigators.[13,19,22,24,25] We expressed results as the number of colony-forming units recovered from each surface sample, rather than as the number of colonies per centimeters squared, because the nonuniform size and shape of the items sampled made it difficult to use a template or Rodac-type contact plates. Nevertheless, we documented that high-touch surfaces were frequently contaminated with a variety of bacteria, including MRSA and VRE.

Although we used the same ATP bioluminescence assay that was utilized in several studies in the United Kingdom, the median RLU values observed in the present study were considerably lower than the mean RLU values reported previously.[19,22] This finding may be related to differences in the types of surfaces sampled and cleaning solutions used in the various studies. The median RLU values observed in phase II of our study were similar to those obtained by Lewis et al.[21] following a modified cleaning protocol. The low degree of correlation between colony counts and ATP readings noted in our study has been reported by others[24,26] and is due to the fact that colony counts detect only viable aerobic bacteria on surfaces, whereas an ATP bioluminescence assay detects all types of organic material present on surfaces.

Phase II was conducted for 2 reasons. We wanted to obtain

a larger sample of observations that reflected the range of ATP readings after daily cleaning on multiple wards by a variety of housekeepers. Also, because the ATP readings obtained during phase I obviously reflected suboptimal cleaning practices, we wanted to establish the level of ATP readings that could be expected when more thorough cleaning was performed. It was for this reason that housekeeper educational sessions were conducted and cleaning personnel were informed in advance that selected rooms would be tested after cleaning. We found that high-touch surfaces were significantly cleaner after daily cleaning during phase II than they were after cleaning during phase I (Figure). Overall, 388 (77%) of 503 surface samples tested after cleaning during phase II had ATP readings of less than 250 RLUs, a recently proposed standard for defining hospital surfaces as clean.[21] Smooth, flat surfaces were more likely than irregular surfaces to yield RLU values of less than 250.

Our study has several limitations. Colony counts were obtained from a small number of rooms and may not reflect the level of bacterial contamination of such surfaces throughout our facility or in other hospitals. Failure to incorporate a neutralizer into culture media may have resulted in an underestimation of the number of bacteria on surfaces. During phase II, financial constraints and limited resources prevented us from performing colony counts. Notifying housekeepers in advance that the room they were about to clean would be monitored could well have resulted in the Hawthorne effect, whereby housekeepers' performance improved only when they knew they were being observed. However, it is of interest to note that an improvement in cleaning practices was sustained throughout phase II and was greater during the latter half of phase II than during the initial half (data not shown). To determine whether the Hawthorne effect accounted for much of the improvement observed during phase II, we are conducting a third phase of the study in which random, unannounced ATP readings will be obtained after rooms have been cleaned, and housekeepers will be given the results of the ATP readings shortly after they have completed cleaning the rooms. In addition, housekeepers deemed by environmental services managers to be the most thorough are being observed, and ATP readings after cleaning are being analyzed in an effort to determine whether the recently proposed breakpoint ATP reading of less than 250 RLUs is a practical criterion for classifying surfaces as clean in acute care settings.[21] Additional studies from multiple healthcare facilities are needed before a standardized ATP bioluminescence breakpoint can be established for defining surfaces as adequately cleaned.

The role of monitoring cleaning procedures in healthcare facilities is just beginning to be understood. A recent study

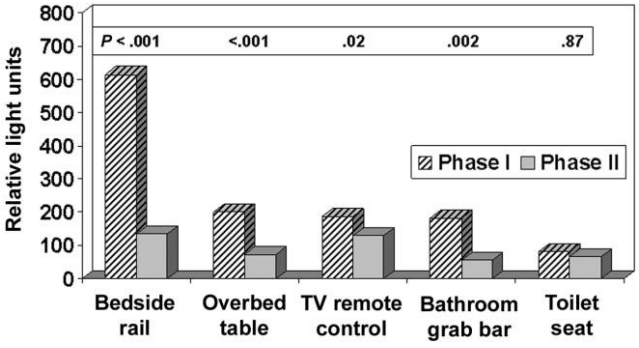

FIGURE. Bar graph of adenosine triphosphate readings, expressed as relative light units, from 5 high-touch surface samples after daily cleaning, during phase I *(striped bars)* and phase II *(solid bars)*. TV, television.

TABLE 3. Phase II Adenosine Triphosphate (ATP) Readings (Expressed as Relative Light Units [RLUs]) of Samples Obtained From 5 High-Touch Surfaces in 105 Patient Rooms, Before and After Daily Cleaning, at the Hospital of Saint Raphael

ATP reading	Bedside rails[a]	Overbed tables[b]	Television remote controls[c]	Bathroom grab bars[d]	Toilet seats[e]
Before cleaning					
<250 RLUs	40/104 (38)	49/104 (47)	44/103 (43)	49/99 (50)	55/100 (55)
250–499 RLUs	21/104 (20)	29/104 (28)	34/103 (33)	23/99 (23)	15/100 (15)
500–999 RLUs	28/104 (27)	16/104 (15)	12/103 (12)	13/99 (13)	9/100 (9)
>1,000 RLUs	15/104 (14)	10/104 (10)	13/103 (13)	14/99 (14)	21/100 (21)
After cleaning					
<250 RLUs	66/103 (64)	90/102 (88)	72/101 (71)	80/99 (81)	80/98 (82)
250–499 RLUs	22/103 (21)	7/102 (7)	20/101 (20)	8/99 (8)	9/98 (9)
500–999 RLUs	8/103 (8)	3/102 (3)	4/101 (4)	3/99 (3)	4/98 (4)
>1,000 RLUs	7/103 (7)	2/102 (2)	5/101 (5)	8/99 (8)	5/98 (5)

NOTE. Data are proportion (%) of surface samples tested.

[a] Median value (range) of 393 (10–17,587) before and 134 (9–3,001) after cleaning ($P < .001$).

[b] Median value (range) of 255.5 (9–4,387) before and 72.5 (12–3,311) after cleaning ($P < .001$).

[c] Median value (range) of 289 (10–130,960) before and 129 (14–9,103) after cleaning ($P < .001$).

[d] Median value (range) of 246 (8–3,480) before and 56 (9–3,259) after cleaning ($P < .001$).

[e] Median value (range) of 195.5 (8–16,313) before and 65.5 (10–5,590) after cleaning ($P < .001$).

demonstrated that, for housekeepers, the combination of education, observation, and feedback resulted in reduced VRE environmental contamination and reduced acquisition of the organism by patients.[3] Marking environmental surfaces with a fluorescent dye, using a black light to detect a residual marker, and providing housekeepers with feedback with regard to the findings has resulted in a greater number of surfaces being cleaned.[7,15,16,27] Of note, a majority of the latter studies did not document that surfaces were in fact cleaner or had less bacterial contamination.[15,16,27] Another study found that the use of a fluorescent marker and feedback based on this monitoring system resulted in surfaces being less contaminated with MRSA and VRE.[7] Of interest, there was no association between the removal of the marker from a specific surface and the likelihood that the surface sample would yield MRSA or VRE on culture. In another study, 33% of toilet samples with no visible residual fluorescent marker were still contaminated with *Clostridium difficile* spores in rooms of patients with *C. difficile*–associated diarrhea.[28] In contrast to fluorescent markers, the ATP bioluminescence assay provides a quantitative measure of the amount of organic material remaining on surfaces after cleaning.

In conclusion, the ATP bioluminescence assay was used in our study to document the level of cleanliness of high-touch surfaces after routine daily cleaning in patient rooms and to study the impact of educational sessions and training on the adequacy of cleaning practices. This assay could also be used to evaluate the efficacy of terminal cleaning procedures. ATP readings can provide real-time feedback to housekeepers regarding their performance, an advantage over the 24–48 hours required to obtain results using microbiological methods. The digital readings obtained using the ATP bioluminescence assay and accompanying data analysis software provide a system for tracking the adequacy of cleaning over time.

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Potential conflicts of interest. J.M.B. has served as a consultant to 3M. All other authors report no conflicts of interest relevant to this article.

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