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# Removing bacteria from hospital surfaces: a laboratory comparison of ultramicrofibre and standard cloths

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**Summary** We compared the ability of ultramicrofibre-woven cloths with conventional cloths moistened with water only, for their ability to remove several types of organisms relevant to hospital-acquired infections from a variety of surfaces in hospitals. We showed that ultramicrofibre cloths consistently outperformed conventional cloths in their decontamination ability, across all surfaces, and irrespective of whether the bacteria were coated on to the surfaces with phosphate-buffered saline (PBS) or PBS containing horse serum to simulate real-life soiling. The ability of the cloths to remove bacteria from surfaces was assessed by contact plating and colony formation, and by swabbing and measurement of ATP bioluminescence. The results suggest potential for use of ultramicrofibre in healthcare environments. Further studies are required, however, to define accurately how these cloths, which are designed to be used without detergent or biocides, might be capable of safe and effective deployment and recycling in the healthcare environment.


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## Introduction

Healthcare-associated infections (HAIs) persist, despite increasing concern and resources allocated to their control. Organisms responsible for such infections may relate to the patient's own flora, or may be acquired either from healthcare staff, or the environment. Controversy persists concerning the relative contribution of the environment to HAI.[1] It is nevertheless clear that some if not most pathogens can contaminate and persist in a viable state in the hospital environment for weeks if not months.[2–4] Many outbreaks have been attributed to such environmental sources, which have to be identified and eliminated for outbreak termination.[5,6] Eliminating such sources relies on physical removal by cleaning, disinfection by biocidal agents, or both.[7]

The physical forces which determine the relative ability of different types of cleaning cloth to remove chemical and particulate matter from surfaces are complex and not easily defined. Accordingly, classical cloth-based cleaning requires disinfectants as well as detergents if pathogens are to be consistently and effectively removed, neutralised, or both.[8,9] The need for agents with disinfectant properties presents its own problems, as microbiocides containing halides, or oxygen-releasing compounds (such as peroxides), or quaternary ammonium compounds can be harmful to health, degrade the built environment, and some, such as triclosan, are implicated in driving antimicrobial resistance.[10–12] Enhanced removal of particulate matter (such as bacteria) from surfaces without having to resort to potentially toxic and destructive biocidal agents would therefore be advantageous not only to local ecology, but also to the structure of the building itself.

Physical removal of dirt and bacteria from the hospital environment usually involves the passage of cloths, or mops, across surfaces. Cloths woven with microfibre (MF) have recently been introduced to the market, and MF mops have been shown to be more effective at surface cleaning in hospital wards than string mops.[9,13] Microfibres are produced by splitting larger fibres of polyester/polyamide composite and by definition weigh less than 1 decitex (1 g/10 000 m). Ultramicrofibres (UMF) are even thinner, and are designed to have a weight of $\leq$0.3 decitex. Unlike conventional yarn-based cleaning materials and systems, UMF cloths are woven from a continuous UMF strand and are designed to be used with low volumes of water containing neither detergent nor biocidal additives. The positively charged fibres remove particles by a combination of static attraction and capillary action. UMF cloths might also conform better to surfaces containing small abrasions invisible to the naked eye, in which bacteria might lodge and remain after passage of a larger conventional wet loop mop fibre. Several UK hospitals are already using this alternative cleaning technology. Unlike the considerable experience and published data concerning microfibre-related removal of bacteria in the field of food safety, we know of no published work relating to the performance of UMF cloths in this respect in the healthcare arena other than that of Moore and Griffith who have recently published a comprehensive laboratory study on the decontamination properties of microfibre cloths.[14,15]

Accordingly, we set out to compare and contrast standard wet loop (J cloths) and UMF cloths for their respective abilities to remove bacteria relevant to HAI from several surfaces currently used in clinical hospital areas.

## Methods

### Materials

Microbiological plates and reagents were purchased from Oxoid Ltd (Basingstoke, UK). The ATP swab test was purchased from Biotrace (Bridgend, UK). Horse serum and bovine serum albumin (BSA) were purchased from Sigma.

### Hard surfaces

We used several different surfaces for these studies: a rough tile (Marmoleum; Forbo Nairn), a smooth tile (Project Vinyl; Forbo Nairn), laminated worktops — both new and worn (aged $>$10 years, taken from a ward in the closed Middlesex Hospital, London) — and stainless steel surfaces identical in grade and finish to those used for University College London Hospitals (UCLH) clean room environments. The surfaces were steam-cleaned (131 °C) before use. At the time of these studies, all of these surfaces were being incorporated into the clinical areas of the new UCLH NHS Trust.

### Cloths

We used UMF cloths manufactured from a composite 80% polyamide/20% polyester fibre (Vikan AB, Viskafors, Sweden). The comparator cloth used was the commercial J cloth, which was used new and

only once. Both types of cloths were moistened with deionised water immediately prior to use.

### Estimation of bacterial contamination by contact plating

The hard surfaces described above were intentionally contaminated with bacterial suspensions whose concentrations were adjusted by McFarland standards, and subsequently validated by culture and calculation according to the method of Miles and Misra.[16] Wild type clinical isolates of meticillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter calcoaceticus var. baumannii* (ACCB), *Klebsiella oxytoca* (*K. oxytoca*) in logarithmic phase growth and spores of *Clostridium difficile* were used in these studies as previously described.[17] Bacterial suspensions were made in phosphate-buffered saline (PBS) with or without 7% BSA, and 100 μL inoculated onto pre-cleaned and marked 100 $cm^2$ areas of each surface and spread using a sterile spreader. After leaving the samples to dry for 2 h at room temperature, the surfaces were then wiped with either UMF or J cloths with a specific crossover motion as recommended by the UMF manufacturer. Bacteria were sampled pre- and post-cleaning using contact plates (tryptic soy agar, Oxoid; 19.6 $cm^2$ contact area) which were placed on the surfaces for 5 s before removal and incubation at 37 °C in either 5% $CO_2$, or in an anaerobic chamber in the case of experiments performed with *C. difficile*, and colony-forming units were counted after 24 or 48 h incubation.

### Estimation of bacterial contamination by measuring surface ATP levels

The hard surfaces were intentionally contaminated with bacteria as described above except in the case of the results presented in Figure 3 where bacterial suspensions in PBS with 10% horse serum were applied three times at two-hourly intervals and then allowed to dry overnight to simulate a soiled surface not cleaned for 16 h. After wiping with UMF or J cloths as described above, the surfaces were swabbed and ATP levels assessed as described below.

### Biotrace ATP bioluminescence assay

The assay was performed as described by the manufacturer (Biotrace, Bridgend, UK). Briefly, swabs were removed from their containers and drawn in a defined and consistent pattern (up and down, then side to side while rotating the swab) across the area to be sampled. The swabs were then reinserted into their containers and allowed to react with the reagents in the cuvette for 10 s. The swabs were immediately placed into the Biotrace hand-held luminometer and the relative light unit (RLU) reading on the display was recorded. In order to avoid variability, all swabbing and readings were made by the same operator.[18]

### Statistical analyses

All statistical analyses were carried out in STATA 9.0 and tests of significance were reported significant if $P < 0.05$. We used (parametric) *t*-tests and (non-parametric) Mann–Whitney tests to compare differences in cfu and RLUs for the two cleaning methods (J cloths and UMF cloths) after cleaning.

## Results

Table I shows that UMF cloths are considerably more effective than J cloths at removing MRSA, *Acinetobacter*, *K. oxytoca* and spores of *C. difficile* (applied in PBS and allowed to dry) from all three of the work surfaces tested. In most cases, cleaning with UMF cloths, but not J cloths, resulted in complete, or almost complete, removal of culturable bacteria or *C. difficile* spores from all three surfaces. Importantly, UMF cloths were particularly effective on old laminate surfaces where the bacteria are most likely to be hidden away in micro-fissures on the used surface. In this experiment, ACCB and to some extent MRSA were particularly poorly removed by J cloths in contrast to UMF cloths.

In the healthcare environment, bacteria are often found on surfaces in association with organic matter. Accordingly, we performed similar experiments with two different seeding densities of bacteria suspended in PBS containing 7% BSA that were applied to four different surfaces commonly used in hospitals. Table II again clearly demonstrates that UMF cloths are more effective than J cloths in removing bacteria from all four surfaces regardless of the number of bacteria seeded. In contrast to the results in Table I, in the presence of 7% BSA *K. oxytoca* was the bacterium most poorly removed by J cloths whereas UMF cloths were highly effective.

In addition to the standard microbiological methods we also used ATP bioluminescence to compare the ability of UMF and J cloths to clean contaminated hospital surfaces. We calibrated the Biotrace ATP assay by directly inoculating the swabs with MRSA, ACCB and *K. oxytoca*. The RLUs

**Table I** Removal of bacteria or bacterial spores from three hospital surfaces using either J cloths (JC) or ultramicrofibre (UMF) cloths

| Target organism | No. of bacteria seeded | New laminate high touch surface (cfu) | | | Old laminate high touch surface (cfu) | | | Steel tile (cfu) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-clean | Post-JC clean | Post-UMF clean | Pre-clean | Post-JC clean | Post-UMF clean | Pre-clean | Post-JC clean | Post-UMF clean |
| MRSA | $2 \times 10^6$ | >500 | 36 | 0 | >500 | 215 | 0 | >500 | 178 | 0 |
| | | >500 | 232 | 2 | >500 | 229 | 0 | >500 | 100 | 0 |
| | | >500 | 91 | 0 | >500 | 251 | 1 | >500 | 119 | 0 |
| ACCB | $2 \times 10^6$ | >500 | 462 | 3 | >500 | 253 | 0 | >500 | 158 | 7 |
| | | >500 | >500 | 7 | >500 | 340 | 0 | >500 | 248 | 12 |
| | | >500 | >500 | 4 | >500 | 364 | 0 | >500 | 435 | 14 |
| *Klebsiella oxytoca* | $2 \times 10^6$ | >500 | 40 | 0 | 481 | 2 | 0 | >500 | 14 | 0 |
| | | >500 | 22 | 0 | >500 | 12 | 0 | >500 | 14 | 0 |
| | | >500 | 34 | 0 | >500 | 14 | 0 | >500 | 38 | 0 |
| *Clostridium difficile* spores | $1.5 \times 10^4$ | 55 | 1 | 0 | 54 | 2 | 0 | 53 | 1 | 0 |
| | | 73 | 0 | 0 | 72 | 0 | 0 | 61 | 1 | 0 |
| | | 76 | 2 | 0 | 62 | 1 | 0 | 59 | 2 | 0 |
| Uninoculated background | — | 2; 3; 0 | — | — | 1; 0; 0 | — | — | 1; 0; 1 | — | — |

MRSA, meticillin-resistant *Staphylococcus aureus;* ACCB, *Acinetobacter calcoaceticus var. baumannii*.
Triplicate readings at each experimental point are shown since readings >500 cannot be used when calculating mean ± SD values.

**Table II** Removal of bacteria suspended in 7% bovine serum albumin from four hospital surfaces using either J cloths (JC) or ultramicrofibre (UMF) cloths

| Target organism | No. of bacteria seeded | Smooth tile (cfu) | | | Rough tile (cfu) | | | New laminated worktop (cfu) | | | Steel tile (cfu) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-clean | Post-JC | Post-UMF | Pre-clean | Post-JC | Post-UMF | Pre-clean | Post-JC | Post-UMF | Pre-clean | Post-JC | Post-UMF |
| MRSA | $1.2 \times 10^5$ | 386 ± 14 | 22 ± 2 | 0 | 469 ± 22 | 44 ± 2 | 1 ± 0 | 352 ± 23 | 6 ± 2 | 0 | 212 ± 3 | 58 ± 4 | 0 |
| | $1.2 \times 10^6$ | >500[a] | 38 ± 3 | 0 | >500[a] | 83 ± 3 | 2 ± 1 | >500[a] | 6 ± 3 | 1 + 1 | >500[a] | 29 ± 4 | 0 |
| ACCB | $5 \times 10^4$ | 310 ± 9 | 27 ± 4 | 0 | 327 ± 8 | 14 ± 1 | 0 | 296 ± 9 | 2 ± 1 | 0 | 214 ± 11 | 21 ± 2 | 0 |
| | $5 \times 10^5$ | >500[a] | 45 ± 3 | 0 | >500[a] | 17 ± 2 | 0 | >500[a] | 3 ± 1 | 0 | >500[a] | 37 ± 3 | 0 |
| *Klebsiella oxytoca* | $6 \times 10^4$ | 289 ± 11 | 132 ± 3 | 0 | 389 ± 14 | 61 ± 2 | 0 | 239 ± 13 | 10 ± 2 | 0 | 312 ± 10 | 38 ± 5 | 0 |
| | $6 \times 10^5$ | >500[a] | 154 ± 7 | 0 | >500[a] | 95 ± 6 | 1 + 1 | >500[a] | 58 ± 1 | 0 | >500[a] | 97 ± 8 | 0 |

MRSA, Meticillin-resistant Staphylococcus aureus; ACCB, Acinetobacter calcoaceticus var. baumannii.
Results shown are mean ± SD of triplicate readings.
[a] In each case all three readings were >500.

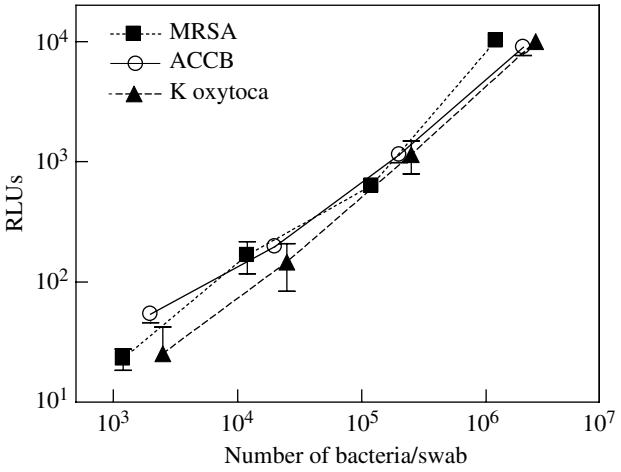


**Figure 1** Validation of the Biotrace ATP bioluminescence kit. Biotrace ATP swabs were directly inoculated with meticillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter calcoaceticus var. baumannii* (ACCB) or *Klebsiella oxytoca*. The swabs were analysed by the luminometer which expresses the ATP of the lysed bacteria content as relative light units (RLUs). The results shown are the mean ± SD of triplicate readings. The mean background reading of swabs wetted with phosphate-buffered saline + 10% horse serum with no bacteria (11 ± 3, $N = 3$) was subtracted from all readings.

for all three bacteria were concentration dependent (Figure 1). The limit of sensitivity was ~$10^3$ organisms for each bacterial strain.

We used the Biotrace ATP assay to assess bacterial contamination on surfaces commonly used in hospitals. As shown in Figure 2, contamination of the four surfaces with bacteria in PBS alone resulted in consistent pre-clean RLUs for all three bacterial strains. In each case, UMF cloths were significantly more effective than J cloths, particularly in the case of MRSA (Figure 2a) and *K. oxytoca* (Figure 2c) on all surfaces.

Healthcare surfaces are often soiled with organic matter that may be left uncleaned or partially cleaned for variable periods of time. We simulated this 'real-life' scenario by applying at two-hourly intervals three 'coats' of bacteria suspended in PBS with 10% horse serum followed by an overnight drying period. The results in Figure 3 show substantially increased (40–120-fold) RLU pre-clean readings compared with those in Figure 2 for all three bacteria on all four surfaces; steel tiles (surface 2) and rough floor tiles (surface 4) had the highest RLUs with all three strains. Despite the high level of bacterial contamination, both UMF and J cloths were effective at reducing RLUs, although again UMF cloths were significantly more effective ($P < 0.001$) in every case.

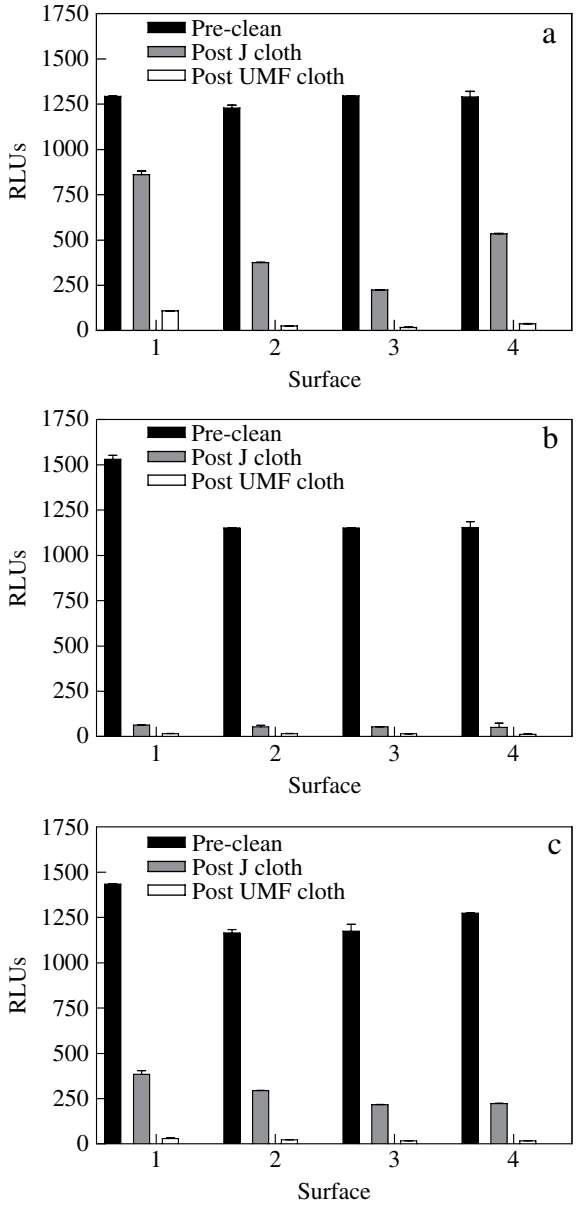


**Figure 2** Removal of bacteria from four hospital surfaces using either J cloths or ultramicrofibre (UMF) cloths: single application without serum. (a) Meticillin-resistant *Staphylococcus aureus* ($1.2 \times 10^5$); (b) *Acinetobacter calcoaceticus var. baumannii* (ACCB) ($5 \times 10^5$); (c) *Klebsiella oxytoca* ($6 \times 10^5$). The bacteria were applied in phosphate-buffered saline and allowed to dry for 1 h. The surfaces used were: 1, laminate worktop; 2, steel tile worktop; 3, smooth vinyl floor tile; 4, rough linoleum floor tile. The surfaces were swabbed and the swabs were analysed by the luminometer which expressed the ATP of the lysed bacteria content as relative light units (RLUs). Background RLUs on uncontaminated areas of the surfaces were: 1: 55 ± 5; 2: 50 ± 2; 3: 42 ± 4; 4: 41 ± 3. The results shown are the mean ± SD of triplicate readings. Univariate analysis of post-clean RLUs for J cloth vs UMF are all significantly different ($P < 0.0001$) except for ACCB on surface 2 ($P = 0.0021$) and surface 4 ($P = 0.04$).

## Discussion

The data presented here clearly demonstrate that when compared with J cloths, UMF cloths consistently remove a larger proportion of organisms responsible for HAI from several intentionally contaminated surfaces relevant to healthcare, irrespective of organism type.

In many cases, passage of UMF cloth over surfaces seeded with $2 \times 10^6$ organisms resulted in total bacterial removal. This superior performance, often leading to complete absence of detectable bacteria on the various surfaces, persisted in experiments designed to mimic protein-rich soiling, and was seen across all types of surface, whether rough or smooth. This finding is impressive when one considers the degree of adherent bioloading subsequent to deposition of bacteria in 10% serum, where RLU values were ~100 times higher than those obtained without a source of nutrient being present (Figures 2 and 3). These results differ significantly from those of Moore and Griffith who compared the decontamination properties of six different microfibre (MF) cloths for *S. aureus* inoculated on steel surfaces.[15] These authors noted, however, significant differences in performance between different MF cloths, both for decontamination ability and subsequent propensity for release of bacteria from the fibres. We have confirmed that all six cloths used in that study were MF (Dr G. Moore, personal communication), as opposed to UMF. It is therefore possible that these divergent results can be explained by the very different structure and size of the fibres in the individual cloths used in the two studies. Like Moore and Griffith we conclude that different MF cloths (and presumably UMF cloths) perform very differently with respect to both removal and retention of bacteria. We are accordingly now engaged in a comparative study of MF and UMF performance to further define those elements which confer performance on the removal and retention of bacteria.

This laboratory study demonstrates the potential for this particular brand and physical make-up of UMF cloth to remove most if not all bacteria from a variety of surfaces present in many UK NHS hospitals, without the need for either biocide or detergent. We acknowledge that such absence of biocide implies risk; organisms picked up by moist UMF make used UMF cloths potential sources of viable organisms — a point highlighted by Moore and Griffith.[15] In this respect, a fundamental issue with microfibre relates to its incompatibility with chlorine-based products that are currently widely used in the hospital environment for mitigation of both

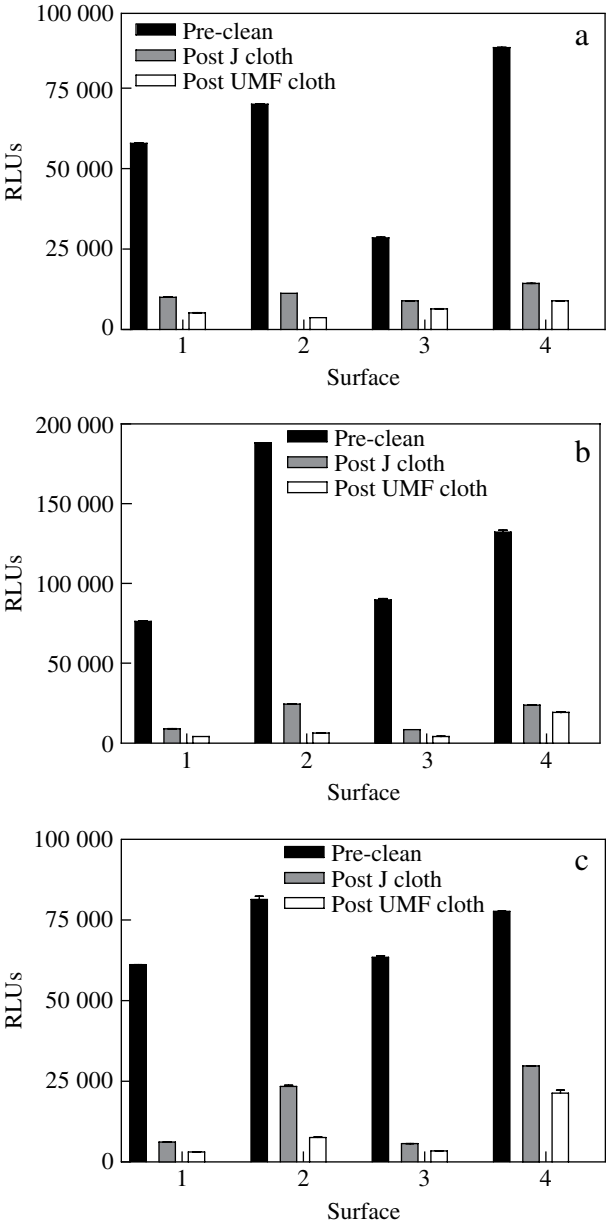


**Figure 3** Removal of bacteria from four hospital surfaces using either J cloths or ultramicrofibre (UMF) cloths: Simulation of 'real-life' soiling. (a) Meticillin-resistant *Staphylococcus aureus* ($1.2 \times 10^5$); (b) *Acinetobacter calcoaceticus var. baumannii* ($2 \times 10^5$); (c) *Klebsiella oxytoca* ($2.5 \times 10^5$). The bacteria were applied in phosphate-buffered saline containing 10% horse serum three times at two-hourly intervals and allowed to dry overnight to simulate a soiled surface not cleaned for 16 h. The surfaces used were: 1, laminate worktop; 2, steel tile worktop; 3, smooth vinyl floor tile; 4, rough linoleum floor tile. The surfaces were swabbed and the swabs were analysed by the luminometer which expresses the ATP of the lysed bacterial content as relative light units (RLUs). The results shown are the mean ± SD of triplicate readings. Multivariate analysis of post-clean RLUs for J cloth vs UMF are all significantly different ($P < 0.0001$).

bacterial and viral HAIs. We have, however, identified an alternative decontamination system based on highly charged copper-based compounds.[17] We are in the process of investigating the application and decontamination performance of UMF in real time in the hospital environment.

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**Conflict of interest statement**
None declared.

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