Comparison of Floor Cleaning and Disinfection Processes in a Research Animal Facility

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Floor cleaning and disinfection are essential components of maintaining animal health status and meeting regulatory requirements in research vivaria. However, best practices for method, frequency, and evaluation techniques have not been established. Reuse of cotton string mop and bucket systems has been implicated in spreading contamination in the human hospital setting. We evaluated 4 different combinations of disinfectant and mop systems commonly used in rodent vivaria. Eight housing rooms were mopped a total of 4 times using one of the following methods: quaternary ammonium compound (QUAT) and cotton string mop (QC), QUAT and microfiber mop (QM), hydrogen peroxide disinfectant (HPD) and cotton string mop (HC), or HPD and microfiber mop (HM). ATP and RODAC samples of the floor were taken before and after mopping. The time to mop each room, floor drying time, and the amount of disinfectant used were recorded. The QC method was associated with significantly more bacterial contamination while all other methods significantly reduced bacterial contamination. The QC method performed significantly worse in reducing bacterial contamination as compared with all other methods being significantly more effective at reducing ATP levels than the QC and QM methods. Costs were similar for the QC, QM, and HM methods. The results of this study indicate that reuse of cotton string mop heads with QUAT increases floor contamination while HPD is effective for up to 3 reuses. Single use microfiber mops were effective with both QUAT and HPD but did not result in more effective cleaning or disinfection than cotton string mops.

Abbreviations and Acronyms: HPD, hydrogen peroxide-based disinfectant; HC, hydrogen peroxide disinfectant and cotton string mop; HM, hydrogen peroxide disinfectant and microfiber mop; QUAT, quaternary ammonium compound; QC, QUAT and cotton string mop; QM, QUAT and microfiber mop; RLU, relative light units

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Introduction

The Guide for the Care and Use of Laboratory Animals states that "All components of the animal facility, including animal rooms and support spaces (that is, storage areas, cage-washing facilities, corridors, and procedure rooms) should be regularly cleaned and disinfected as appropriate to the circumstances and at a frequency based on the use of the area and the nature of likely contamination."20 This performance-based standard allows the institution to determine the method, frequency, and evaluation techniques used to assess cleaning and disinfection. Floor cleaning and disinfection in rodent vivaria are most frequently accomplished by sweeping and mopping regularly. Several types of mops are available including cotton string mop and bucket, disposable microfiber mops, and reusable microfiber mops. In the human hospital setting, much research has been devoted to environmental cleaning and disinfection techniques, as well as evaluation methods, in an effort to limit hospital acquired infections.^{10,18} This research has largely led to replacement of cotton string mop and bucket systems with microfiber mops or strict laundering and sanitation of mopping materials before reuse. Mop head reuse can lead to bacterial growth and subsequent spreading of contamination during cleaning procedures.^{10,25,30,32} Microfibers are thin polyester and polyamide fibers that are more absorbent than cotton. They are

positively charged and attract negatively charged particles such as dirt and microorganisms. In addition, microfibers can penetrate the microscopic pores in most flooring material.^{22,29} Other benefits of microfiber mops include reduced chemical and water use, reduced cleaning time, and reduced occupational exposure and injuries.^{21,24,25,30} For these reasons, microfiber mops are generally considered to be more effective for floor cleaning.

At our institution, animal rooms were swept and mopped twice a week using a cotton string mop and diluted quaternary ammonium compound (QUAT) disinfectant. Visibly soiled mop heads were laundered, but this subjective approach resulted in variable replacement schedules. Based on literature from the human hospital setting, we wanted to validate the effectiveness of our floor sanitation protocols. Initial sampling of mop materials in one animal suite using replicate organism detection and counting (RODAC) plates revealed colonies too-numerous-tocount on the mop head, no colonies on the mop bucket interior, and substantially more colonies on the floor after mopping. These preliminary findings suggested that current practices were potentially spreading microorganisms throughout our facility via the mop head. In researching alternative mopping systems, we learned that QUAT disinfectants may bind to microfiber and cotton, thereby reducing the QUAT concentration of the solution.^{6,14} To control for this complication, we evaluated a different class of disinfectant (an oxidant) in this study.

The purpose of this current study was to compare the efficacy of 4 different combinations of disinfectant and mop systems for cleaning rodent housing room floors. Cleaning and disinfection

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Materials and Methods

microfiber mops would provide more effective cleaning and dis-

infection than cotton string mops, regardless of disinfectant used.

A total of 8 rodent housing rooms with approximately 15-y-old epoxy flooring were selected for the study based on the floor condition (completely intact with no cracks or stains) and similar numbers of racks, cages, and investigators in each room to approximate similar foot traffic. In the selected vivarium, housing areas are organized into suites containing 4 animal housing rooms and 2 procedure rooms with a shared corridor. Animals held in the selected suites all had the same health status. All housing rooms used IVC racks with automatic water valves, biosafety cabinets for cage changes, and irradiated feed. Suite A was considered to be sterile housing, and IVC cages for this area were autoclaved prior to use. Two of the housing rooms in suite A measured $6.6 \text{ m} \times 4.8 \text{ m}$ while the others measured 6.6 m \times 4.5 m. All housing rooms in suite B measured 6.7 m \times 4.9 m. A single bidirectional corridor connects suites A and B. PPE is required to enter animal housing rooms and includes a disposable gown, gloves, and mask. Shoe covers are not used in this facility. Floor cleaning procedures before the study began were the same for both suites and included twice weekly sweeping and mopping with a QUAT disinfectant. Each animal room had a dedicated broom; the mop head, mop bucket, and disinfectant were shared among rooms within a suite. After mopping, water in the mop bucket was discarded, and the mop was hung to dry in a dedicated janitorial closet until its next use.

To control variability in mopping technique, 2 designated personnel performed all mopping procedures during the 2-wk study period, adhering to the same twice weekly cleaning schedule for a total of 4 mopping trials in each suite. Each room in a suite was assigned one of the following experimental methods: QUAT and cotton string mop (QC), QUAT and microfiber mop (QM), hydrogen peroxide-based disinfectant (HPD) and cotton string mop (HC), and HPD and microfiber mop (HM). Rooms were mopped with QUAT using Process NPD One Step Disinfectant (Steris Life Sciences, Mentor, OH) diluted to 0.4% based on manufacturer instructions. One gallon of diluted QUAT was used in QC rooms, whereas diluted QUAT was placed into a squirt bottle for use with the microfiber mop in QM rooms. All QUAT dilutions were confirmed to be 600 ppm using Hydrion Quat Check 1000 Strips (Micro Essential Laboratory, Brooklyn, NY). Rooms assigned to HPD were mopped using Peroxigard Ready-to-Use solution (Lighthouse Life Sciences, Woburn, MA), which requires no dilution or preparation. One gallon of HPD was used in HC rooms whereas HPD in a squirt bottle was used in HM rooms. All rooms were swept immediately before mopping and experimental conditions within rooms were constant for the duration of the study. All cotton string mop heads (Rubbermaid, Atlanta, GA) were new at the beginning of the study and were dedicated to each room so they could be reused throughout the study (hung to dry after each mopping trial). All mop buckets (Rubbermaid) were visibly clean and thoroughly scrubbed with soap and water before the start of the study. Each mop bucket was dedicated to a room and reused throughout the study, with a water rinse after each use.

A microfiber mop (Trust Mop System, Lighthouse Life Sciences) with disposable mop pads (Trust High Absorbency Single-Use Microfiber Mop Pads, Lighthouse Life Sciences) was used; pads were disposed of after each use based on manufacturer instructions. All rooms were mopped in an S-pattern without moving racks or other items in the room. In the QC and HC methods, the back half of the room was mopped, then the mop was submerged in disinfectant and wrung, and then the front half of the room was mopped. The volume of disinfectant used in QM and HM methods was recorded. The time to mop each room and the time needed for each room to dry completely were recorded with a stopwatch.

For sample collection, the room was divided into 6 quadrants and samples were taken roughly from the center of each quadrant immediately before and as soon as the floor was completely dry after mopping. Samples were taken from approximately the same location on the floor before and after mopping. For each sampling site, ATP and RODAC samples were taken immediately adjacent to each other. Twelve RODAC samples (Trypticase Soy Agar with Lecithin and Polysorbate 80, Becton, Dickinson, and Company, Franklin Lakes, NJ) were taken in each room (6 quadrants sampled before and after mopping) for a total of 384 RODAC samples. Six ATP swabs (UltraSnap Surface ATP Swab, Hygenia, Camarillo, CA) were taken in each room (3 quadrants sampled before and after mopping) for a total of 192 ATP swabs. ATP swabs were used according to manufacturer instructions and were read within one minute after collection using a luminometer (SystemSURE PLUS ATP Measurement System, Hygenia) to detect organic matter. Positive and negative controls were included at the beginning of the study before use. Sampling of the suite hallway served as the positive control while mimicking the actions and duration of sampling without touching the floor's surface served as the negative control. RO-DAC plates were used according to manufacturer instructions and incubated for 24 h before reading to detect growth of live microorganisms. Colonies were counted manually by a single observer. Colonies too-numerous-to-count were recorded as 200 for statistical analyses.

Data were analyzed with Prism 9.4.0 statistical software (GraphPad Software, San Diego, CA). Descriptive statistics were used to summarize ATP and RODAC data. A Wilcoxon matched-pairs signed-rank test was used to compare relative light units (RLU) and colony-forming units (cfu) values (median and IQR) before and after mopping within each experimental condition (data combined across trials and suites). The percent change in RLU and cfu was calculated for paired samples collected before and after mapping for each individual sampling location within each experimental condition. These data were not normally distributed according to the D'Agostino and Pearson test and could not be normalized. Therefore, Kruskal-Wallis and Dunn multiple comparisons tests were performed using percent change values to identify significant differences between methods. Because no statistically significant differences were detected between suites, data from both suites were pooled for statistical analysis. The cfu data from 2 instances of suspected RODAC plate contamination were not included in the analyses. A Welch t test was used to compare floor mopping time and volume of disinfectant. Kruskal-Wallis and Dunn multiple comparisons tests were used to compare floor drying times because these data were not normally distributed according to the D'Agostino and Pearson test and could not be normalized. P values less than 0.05 were considered statistically significant in all analyses.

Results

ATP data. Mean RLU with the SD for each method before and after mopping are reported separately for each suite in Table 1. Comparison of before and after mopping RLU data revealed statistically significant differences for the QM (P = 0.0008), HC (P < 0.0001), and HM (P < 0.0001) methods but not for the QC method (Figure 1). In all cases, RLU values were significantly lower after mopping. When data from each trial were combined and compared across methods (Figure 2), a statistically significant difference in RLU percent reduction was detected between the QC and HC methods (P < 0.0001), the QC and HM methods (P = 0.0002), the QM and HC methods (P = 0.0043), and the QM and HM methods (P = 0.0498). In each case, the HC and HM methods had greater percent reductions in RLU. Analysis of data from the first trial alone revealed no statistically significant differences between any of the methods. When the subsequent

Table 1. Mean RLU \pm 1 SD of 4 mopping methods before and after mopping across 4 trials in 2 rodent housing suites

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Method	Pre-RLU mean ± 1 SD	Post-RLU mean ± 1 SD		
Suite A QC	56 ± 34	95 ± 88		
Suite B QC	598 ± 273	620 ± 284		
Suite A QM	401 ± 292	252 ± 174		
Suite B QM	166 ± 79	120 ± 33		
Suite A HC	143 ± 268	26 ± 27		
Suite B HC	53 ± 39	20 ± 10		
Suite A HM	179 ± 78	99 ± 90		
Suite B HM	219 ± 118	87 ± 61		
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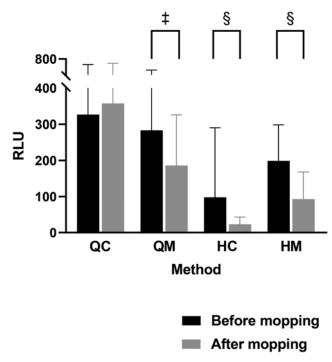


Figure 1. Number of RLU (mean ± 1 SD, n = 24 per method) before and after mopping rodent housing rooms as follows: QUAT and cotton string mop (QC), QUAT and microfiber mop (QM), hydrogen peroxide-based disinfectant (HPD) and cotton string mop (HC), and HPD and microfiber mop (HM). Data were combined over 4 trials in 2 different housing suites. Significant differences (‡, $P \le 0.001$; §, $P \le 0.0001$) were found between values obtained before and after mopping based on the Wilcoxon matched-pairs signed-rank test.

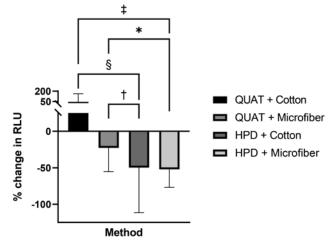


Figure 2. Effectiveness of organic debris removal from rodent housing room floors using various mopping methods as measured by percent change in ATP levels (mean ± 1 SD, n = 24 per method). Mopping methods were QUAT and cotton string mop, QUAT and microfiber mop, hydrogen peroxide-based disinfectant (HPD) and cotton string mop, and HPD and microfiber mop. Data were combined over 4 trials in 2 different housing suites. Groups were significantly different (*, $P \le 0.05$; \dagger , $P \le 0.01$; \ddagger , $P \le 0.001$; \$, $P \le 0.001$) based on the Kruskal-Wallis test followed by the Dunn multiple comparisons test.

3 trials were analyzed separately, the QC and HC methods (P = 0.0057 Trial 2, P = 0.01 Trial 3) and the QC and HM methods (P = 0.0173 Trial 2, P = 0.0478 Trial 3, P = 0.033 Trial 4) were significantly different from each other, except for trial 4 in which only QC and HM differed significantly. In all instances, the QC method had the smallest percent reductions in RLU.

RODAC data. Mean cfu with the SD and median cfu with the IQR for each method before and after mopping are reported separately for each suite (Table 2). Comparison of paired before and after mopping cfu data revealed statistically significant differences (P < 0.0001) for all methods (Figure 3). In the QC method, cfu values after mopping were higher than those measured before mopping. For all other methods, cfu values were lower after mopping. When data from each trial were combined and compared across methods, a statistically significant difference (P < 0.0001) in cfu percent reduction was detected between QC and the other methods, with the QC method having smaller percent reductions in cfu (Figure 4). When the first trial was analyzed separately, the only significant difference (P = 0.0032) was between the QM and HC methods, with the QM method having a greater percent reduction in cfu. When the subsequent 3 trials were analyzed separately, the same pattern of significance was seen as in the combined data except for trial 3, in which QM and HC were also significantly different (P = 0.0279) with the HC method having a greater percent reduction in cfu (Trial 2: P = 0.0105 [QC compared with QM], P < 0.0001 [QC compared with HC, QC compared with HM]; Trial 3: P = 0.0191 [QC compared with QM], P < 0.0001 [QC compared with HC], P = 0.0003[QC compared with HM]; Trial 4: P = 0.0151 [QC compared with QM], P < 0.0001 [QC compared with HC, QC compared with HM]). When the percent reduction in cfu was analyzed over time within a method, a statistically significant difference was detected between the first trial and the subsequent 3 trials in QC (*P* = 0.0466 [Trial 1 compared with 2], *P* < 0.0001 [Trial 1 compared with 3, 1 compared with 4]) and HC (P = 0.0116[Trial 1 compared with 2], P < 0.0001 [Trial 1 compared with 3], P = 0.0002 [Trial 1 compared with 4]). For the QC method, the first trial had greater percent reductions than subsequent trials

Table 2. Mean cfu \pm 1 SD and median \pm IQR of 4 mopping methods before and after mopping across 4 trials in 2 rodent housing suites

Method	CFU before (mean ± 1 SD)	CFU after (mean ± 1 SD)	CFU before (median ± IQR)	CFU after (median ± IQR)
Suite A QC	31 ± 39	106 ± 91	16 ± 14	89 ± 183
Suite B QC	21 ± 15	149 ± 84	16 ± 19	200 ± 122
Suite A QM	12 ± 8	2 ± 2	10 ± 12	1 ± 3
Suite B QM	23 ± 18	6 ± 4	17 ± 24	6 ± 5
Suite A HC	26 ± 42	4 ± 8	9 ± 25	1 ± 4
Suite B HC	17 ± 16	2 ± 4	14 ± 12	0 ± 2
Suite A HM	14 ± 10	3 ± 5	12 ± 16	1 ± 3
Suite B HM	17 ± 12	2 ± 3	13 ± 10	1 ± 2

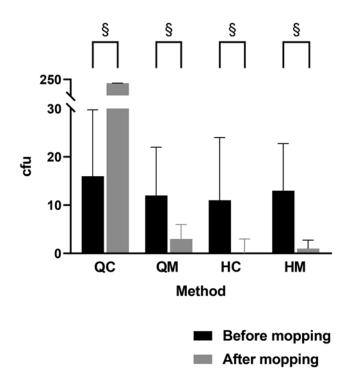


Figure 3. Number of cfu (median \pm IQR, n = 47-48 per method) before and after mopping rodent housing rooms as follows: QUAT and cotton string mop (QC), QUAT and microfiber mop (QM), hydrogen peroxide-based disinfectant (HPD) and cotton string mop (HC), and HPD and microfiber mop (HM). Data were combined over 4 trials in 2 different housing suites. Significant difference (§, $P \le 0.0001$) were found between values obtained before and after mopping based on the Wilcoxon matched-pairs signed-rank test.

(that is, performance was worse over time). In the HC method, the first trial had a smaller percent reduction than subsequent trials (that is, it performed better over time). No significant differences were detected over time for the QM and HM methods.

Efficiency data. The mean time to mop one animal room using a cotton string mop was 515 s (range: 470 to 602 s), including the time to fill the bucket and clean it afterwards. The mean time to mop using a microfiber mop was 410 s (range: 355 to 459 s), including the time to apply and dispose of the microfiber pad. Mopping using a microfiber mop took significantly less time than using a cotton string mop (P < 0.001). The mean floor drying times for each method were 708 s (QC, range: 480 to 904 s), 950 s (QM, range: 682 to 1440 s), 529 s (HC, range: 326 to 752 s), and 553 s (HM, range: 224 to 751 s). The floor took significantly longer to dry after the QM method as compared with HC

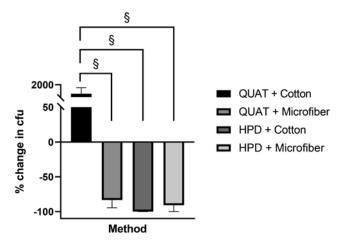


Figure 4. Disinfection effectiveness of rodent housing room floors using various mopping methods as measured by percent change in cfu (median ± IQR, n = 47–48 per method). Mopping methods were QUAT and cotton string mop, QUAT and microfiber mop, hydrogen peroxide-based disinfectant (HPD) and cotton string mop, and HPD and microfiber mop. Data were combined over 4 trials in 2 different housing suites. Groups differed significantly (§, $P \le 0.0001$) based on the Kruskal-Wallis test followed by the Dunn multiple comparisons test.

Table 3. Annual cost comparison of various mopping methods used to mop one animal room twice weekly

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Disinfectant** \$30 \$1,585 \$3 \$68 Staff time*** \$223 \$223 \$178 \$178	Item Cost				Microfiber + HPD
Staff time*** \$223 \$178 \$178	Mopping supplies*	\$123	\$123	\$132	\$131
	Disinfectant**	\$30	\$1,585	\$3	\$68
Total \$377 \$1,932 \$313 \$378	Staff time***	\$223	\$223	\$178	\$178
	Total	\$377	\$1,932	\$313	\$378

*Assumes 12 cotton mop heads used per year and 1 microfiber mop pad per use

**Water costs to dilute the QUAT are not included as the price of water in the Midwest is negligible

***Based on a staff salary of \$15 per hour using average mopping parameters from this study

(P = 0.0054) and HM (P = 0.0211) methods. The mean volumes of disinfectant used with a microfiber mop per room were 343 mL (QUAT, range: 210 to 510 mL) and 164 mL (HPD, range: 71 to 205 mL). Significantly less HPD was used with the microfiber mop than QUAT (P = 0.0004). A cost comparison analysis of the mopping methods is presented in Table 3. The cost to mop a single animal room twice weekly for one year, including all mopping supplies and personnel time for each method is: \$313 (QM), \$377 (QC), \$378 (HM), and \$1,932 (HC). Microfiber mops cost \$9 more than cotton string mops but cost \$45 less to use, making them less expensive overall. In all methods, ready-to-use HPD was more expensive than concentrated QUAT.

Discussion

When data from all trials were combined, RODAC sampling indicated that the QC method was significantly different from all other methods. The positive percent change in cfu with the QC method indicates that cfu *increased* after mopping whereas cfu decreased for all other methods. Comparison of paired cfu data in the QC method also supported a significant increase in cfu after mopping. These findings are consistent with our preliminary data and previous research in the human healthcare setting that has demonstrated that traditional cotton string mop and bucket systems promote

microorganism growth and spread unless mop heads are frequently and appropriately laundered, and mop water is changed often.^{12,32} In hospital settings, the link between environmental contamination from mops and other cleaning materials and patient infection has been well established.^{10,15} The same pattern was not seen for the HC method, a finding that we suspect is due to the stronger disinfection properties of HPD compared with QUAT. As a disinfectant class, oxidants have broader efficacy than denaturants⁷ and the HPD used in this study has a 1-min contact time compared with the 10-min contact time for QUAT. When each trial was analyzed individually, this pattern of significance was not seen during the first trial with a new mop head and only emerged during subsequent trials with mop head reuse. This finding was confirmed by a statistically significant difference between the first and all subsequent trials that used the QC method. The finding that the QC method had greater reductions in cfu with a new mop head is consistent with other studies that evaluated either new or laundered mop heads.^{3,25,27,31}

While the combined ATP data showed similar trends as the RODAC data, no significant differences were found between before and after mopping RLU values for the QC method. The QC and QM methods were also not significantly different from each other. However, both of those methods were significantly different from the HC and HM methods, suggesting that HPD may be more effective at removing organic matter than QUAT regardless of mop type used. While the exact surfactants in each product are proprietary, the manufacturer states that the HPD we used has a strong surfactant profile. However, given the higher variability in RLU values and discrepancies between RLU and cfu data, caution is necessary when interpreting ATP data. ATP data aligned with RODAC results in that differences were not found between any of the methods during the first trial. During subsequent trials, the same ATP findings were found as in the combined data except in the fourth mop trial, in which only the QC and HM methods were significantly different. The smaller ATP sample size for individual trials may explain this outcome. Another possible explanation for this finding and for the high variability in RLU values is that our values may not be in the optimal range of detection for the luminometer used. This issue has been discussed previously, with one study reporting RLU values less than 1,000 to have high variability.^{17,29} The mean of all RLU values in our study was 196 with 98% of RLU values less than 1,000.

Even though the QM method was less effective than the HC or HM methods in terms of RLU reduction, it was nonetheless still significantly better than the QC method for reducing cfu. One possible explanation is that the drying time for the QM method was 241 s longer on average than that of the QC method. A previous study also found longer drying times when QUATs were used with microfiber compared with cotton mops.²⁵ The longer contact time of the QM method could have contributed to its greater reduction in cfu and RLU. The manufacturer recommends a 10-min contact time; all trials using QUAT reached this contact time except for 2 instances in which the floor dried in 8 min and 9 min 17 s after QC mopping. Another explanation for the poor performance of the QM and QC methods as compared with the HC and HM methods is a phenomenon known as QUAT binding, in which the quaternary ammonium compounds bind to cotton and microfibers, reducing the QUAT concentration and therefore the effectiveness. However, these results are highly dependent on the type of QUAT, cotton, and microfiber used.^{6,14,29} To our knowledge, binding has not been documented with HPD. Another possible contributing factor is that disinfectants themselves can affect RLU values. Two

studies have show that QUATs can increase or decrease RLU values; conflicting findings may be due to the use of different materials and methods.^{19,29} Acidic peroxygen sanitizer, a HPD, decreased RLU values in one study.¹⁹

In the current study, microfiber mops were less expensive to use because of the large cost savings they provided in staff time. Other advantages of microfiber mops include lighter mop weight, fewer materials (no need for a bucket and wringer system), reduced chemical use and disposal, reduced water use, and potential construction savings as no janitorial closets and sinks are needed.^{21,24,25,30} These benefits could result in fewer occupational injuries and more sustainable practices. When comparing disinfectant costs, HPD was more expensive than QUAT. However, the ready-to-use formulation of HPD with a microfiber mop is financially comparable and may reduce occupational health concerns related to the dilution and use of QUATs.⁴ The use of concentrated HPD or other disinfectants may significantly reduce disinfectant costs.

Limitations of our study include the well-documented issues related to ATP and RODAC sampling as a proxy for cleanliness and disinfection. RODAC sampling will only detect live, cultivable, aerobic microorganisms. ATP sampling will detect any organic matter but can be affected by the presence of disinfectants and may not efficiently detect gram negative bacteria.^{13,29} Nonetheless, ATP and RODAC testing are standard for evaluation of cleaning and sanitation efficacy.^{1,5,8,9,11,13,16-18,23,25-28,31} Ready-to-use HPD with a string mop and bucket system is substantially more expensive than the concentrated formulation but to avoid introducing additional variables, we opted for the ready-to-use formulation in all HPD methods. Use of concentrated disinfectants would likely still require routine laundering or replacement of the mop head for optimal results; this possibility could be assessed in future research.

The current study confirmed previous research in the human hospital setting indicating that reuse of cotton string mop heads with a QUAT disinfectant without laundering or other processing of the mop head does not clean or sanitize floors but instead increases contamination. The same result was not seen when a cotton string mop was used with HPD, despite reusing a mop head 3 times over a 2-wk period. Therefore, our hypothesis that microfiber mops would provide more effective cleaning and disinfection than cotton string mops regardless of disinfectant was refuted. Microfiber mops with either QUAT or HPD and cotton mops used with HPD both reduced cfu and RLU on rodent vivarium floors.

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