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Extended Sanitation Intervals for Cage Components and Automated Watering Valves: Validation and Cost Analysis

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Although the *Guide* suggests changing rodent cage components every 2 wk, it states that "decreased sanitation frequency may be justified if the microenvironment in the cages, under the condition of use …, is not compromised." The purpose of this study was to evaluate extended sanitation intervals of cage components (automated watering valve, wire bar lid, and filter top) of mouse individually ventilated caging (IVCs) at our institution. We hypothesized that there would be no significant difference in relative light units measured by ATP luminometry of these cage components at the control time point of 14 d as compared with each extended time interval: 28, 56, and 84 d. In addition, for automated watering valves, the study was extended to 168 d. We also hypothesized that time-and-motion studies performed by moving to a sanitation interval of 84 d for all components would result in substantial time and cost savings. The components of a total of 24 cages containing 4 or 5 mice each were swabbed, and an ATP luminometer was used to detect organic matter. We found no significant differences in organic matter load between 14 d and all other time points for all cage components. Our time- and cost-savings analysis found that extending the sanitation interval of cage components from every 2 wk (14 d) to every 3 mo (84 d) for every 10,000 cages would save about 3,000 technician hours annually, for a total annual labor cost savings of about $100,000. This study is the first to validate the extended sanitation interval of automated watering valves and confirms the findings of previous studies that validated the extended sanitation frequency of wire bar lids and filter tops of rodent IVCs. Overall, extending the sanitation frequency of cage components reduces workload of animal care staff without compromising the cage microenvironment.

Abbreviations and Acronyms: IVC, individually ventilated caging; RLU, relative light unit; RODAC, replicate organism detection and counting

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Introduction

The *Guide for the Care and Use of Laboratory Animals* (eighth edition) provides general recommendations for sanitation frequencies of materials in the animal microenvironment, stating that "in general, enclosures and accessories, such as tops, should be sanitized at least once every 2 wk."[5] However, it also states that "decreased sanitation frequency may be justified if the microenvironment in the cages, under the conditions of use …, is not compromised. Verification of microenvironmental conditions may include measurement of pollutants such as ammonia and CO_2, microbiologic load, observation of the animals' behavior and appearance, and the condition of bedding and cage surfaces."[5] The increasing use of individually ventilated caging (IVCs) has led to investigations of extended cage sanitation intervals and increased housing densities.[5] This is due to the fact that IVCs supply HEPA filtered air to each cage microenvironment, resulting in lower temperature, humidity, and concentrations of ammonia and carbon dioxide as compared with static cages.[11] IVCs decrease the risk of cage-to-cage transmission of infectious agents as compared with open top caging[4] and maintain good air quality between cage changes by having continuous air flow. These innovations in rodent housing prompt consideration of extending the sanitation frequency of caging components.

Multiple studies conducted at several institutions have used various methodologies (ATP luminometry,[2,14] microbial culture plates,[3,15] or their combination[13]) to validate an extension of sanitation frequencies for wire bars lids and filter tops in rodent IVC caging to between 60 and 180 d. However, none of these studies duplicated the exact caging setup and husbandry practices used in our facilities, so we opted to validate extended sanitation frequency of wire bar lids and filter tops at our institution. Furthermore, no studies to date have examined an extended sanitation frequency of automatic watering valves on IVC racks. Because automatic watering valves come into direct contact with the animals, they are clearly a component of the cage microenvironment and should be sanitized at regular intervals, as are other caging components.

ATP luminometry measures the amount of organic matter (live and dead) present in a sample by quantifying the amount of ATP present. A surface is swabbed, the sample is exposed to a lysis buffer that releases ATP, and ATP that is present reacts with a light-producing substrate (luciferin) and enzyme (luciferase). When ATP reacts with the luciferase enzyme, it produces light in direct proportion to the amount of ATP present, and this is recorded in relative light units (RLUs). ATP luminometry detects cells and organic contaminants with a strong degree of linear

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predictability.[6,16] This method has been widely used in sanitation monitoring in the food industry, human medical facilities, food animal facilities, and animal research facilities.[1,6,8,12,19] In addition, ATP luminescence is a more efficient method of sanitation monitoring than is replicate organism detection and counting (RODAC) plates because of its ability to detect organic materials (not just live organisms) and rapid assessment of samples (resulting in time and cost savings).[7] For these reasons, we elected to use ATP luminometry to assess the organic debris load of wire bar lids, filter tops, and automatic watering valves in our study.

Extending sanitation frequency of cage components has the potential to save personnel time, decrease facility expenses, and promote more environmentally friendly practices by conserving energy and water usage. These advantages accrue because decreasing the sanitation interval of cage components will likely shorten the time taken to change cages, and also reduce time and conserve energy in cage wash because of the need to process fewer items through the tunnel washer. Therefore, in this study, we also analyzed the time and cost-savings effects of moving from a 2-wk to a 3-mo sanitation interval of cage components. We hypothesized that the levels of organic contamination, as determined by RLUs, would not differ significantly between 14 d and several months of use. We also hypothesized that moving to a 3-mo sanitation interval of cage components would result in significant savings of time and costs.

Materials and Methods

Animals and husbandry. A total of $n = 24$ cages of mice were used for the study. Mice were housed in the University of Chicago ARC facilities RRID:SCR_021806. Cages of adult male and female mice from the program's training colony were used for this study, including C57BL/6, Crl:CD1(ICR), CFW, Crl:NIHBL(S), and various transgenic strains donated by researchers. Cages with 4 to 5 mice, housed by sex, were included on the study, and the housing density was static throughout the study. Cage densities of 4 to 5 mice per cage were used to ensure that the highest caging densities were included while using the largest possible sample size in the training colony. Any cage with a mouse that required euthanasia due to health concerns was excluded from the study.

Mice were housed in solid-bottom polysulfone IVC (19.69 × 30.48 × 16.51 cm; Jag 75 Micro-Barrier IVC, Allentown Caging) at 60 air changes per hour. All cages and cage components (wire bar lids, filter tops, and automated watering valves) were sanitized using a tunnel washer (Basil 6000; STERIS, Mentor, OH), with detergent (Labsan 120; Sanitation Strategies, Holt, MI). To ensure that an appropriate sanitation temperature (180 °F [82.2 °C]) was achieved, a temperature-indicating strip (TempTape 180; Pharmacal Research Laboratories, Naugatuck, CT) was run through the tunnel washer at the start of each day. All cages, cage components, bedding, and enrichment were then autoclaved before use (autoclave Job # 971290; Primus, Orlando, FL) with a sterilization time of 20 min at 252 °F (122.2 °C). Cages contained 1/4-in. corncob bedding (Teklad 7097; Envigo, Indianapolis, IN) and approximately 4 g of specialty shredded paper (Bed-r'Nest; Lab Supply, North Lake, TX) as enrichment. All mice were fed irradiated standard rodent diet (Teklad 2918; Envigo, Indianapolis, IN) and received reverse-osmosis–treated chlorinated water through an automatic watering system (Avidity Science, Waterford, WI). Drinking water was treated with chlorine at 2.0 parts per million and tested weekly to verify chlorine levels. Cage change was performed every 14 d in a class II type A2 biosafety cabinet (NuAire, Plymouth, MN). Mice were transferred to the fresh cage from the base of the tail using forceps soaked in Clidox (Pharmacal, Waterbury, CT), which was the standard of practice at our institution at the time of the study.

Animal rooms were maintained on a 12:12 h light:dark cycle with humidity ranging from 30 to 70% and temperatures ranging from 68 to 76 °F (20 to 24 °C) in compliance with the *Guide*.[5] Mice were checked daily by the animal care staff to assess their health status and the availability of appropriate food, water, and cage conditions.

Routine colony health monitoring was performed quarterly by exhaust dust testing via PCR, as described previously.[10] Excluded agents were Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse parvoviruses, reovirus, epizootic diarrhea of infant mice, mouse encephalomyelitis virus, ectromelia virus, lymphocytic choriomeningitis virus, murine adenovirus, murine cytomegalovirus, K virus, polyoma virus, mouse thymic virus, hantavirus, lactate dehydrogenase-elevating virus, *Filobacterium rodentium*, *Mycoplasma pulmonis*, *Salmonella* spp., *Citrobacter rodentium*, *Clostridium piliforme*, *Streptobacillus moniliformis*, *Corynebacterium kutscheri*, and endo- and ectoparasites including *Hymenolepis* spp., *Giardia muris*, *Encephalitozoon cuniculi*, *Myobia musculi*, *Myocoptes musculinus*, *Radfordia affinis*, *Psoregates simplex*, *Syphacia* spp., and *Aspiculuris tetraptera*.

All animal care and use was conducted in accordance with federal polices and guidelines and was approved by the University of Chicago's IACUC. The University of Chicago has a Public Health Service (PHS) assurance with Office of Laboratory Animal Welfare (OLAW) and is AAALAC accredited.

Study design. The change frequency of caging bottoms for all 24 cages was kept constant at 14-d intervals throughout the study. However, wire bar lids, filter tops, and automatic watering valves were not changed for the duration of the experiment, which was 84 d, or approximately 3 mo. Because many institutions sanitize their automatic watering valves at extended intervals, and some institutions use valves that do not detach from the rack, we elected to extend our testing interval for the valves. We continued to swab a subset of the automatic watering valves ($n = 16$ cages total) at 4-, 5-, and 6-mo time points. When cage bottoms were changed (every 14 d), the automatic watering valve was wiped down with a CaviWipe (Metrex, Orange, CA) before returning the cage to the rack. We opted to wipe the valve with a CaviWipe in the study because that was our standard of practice at the time of study. Furthermore, mechanical wiping helps to prevent bedding and food from building up and causing possible leakages or blockages of automatic water flow.

ATP luminometry. ATP swabs (UltraSnap Surface ATP Test; Hygiena, Camarillo, CA) were used to collect organic debris on the wire bar lids, filter top, and automated watering valve at 14, 28, 56, and 84 d. As mentioned above, a subset of automated watering valves was also swabbed at 112, 140, and 168 d. A consistent 4- × 4-in. area at near right (when facing the cage on the IVC rack) was swabbed on both the outside of the wire bar lid and inside of the filter top; swabbing was performed inside the biosafety cabinet. This location was chosen because it allowed swabbing of a flat 4- × 4-in. area of the wire bar lid that was not impeded by food or a water bottle (see Figure 1A). Swabbing was performed in 3 directions with 10 passes each: vertically, horizontally, and diagonally as shown (Figure 1B). The swab was rotated between gloved fingers throughout swabbing to increase surface area contact. For the automated watering valve, the swab was passed 10 times around the circumference of the valve, working from the base to the tip, with the final swab being of the front portion of the valve (Figure 1C). The valve swabbing occurred while the valve was connected to the rack

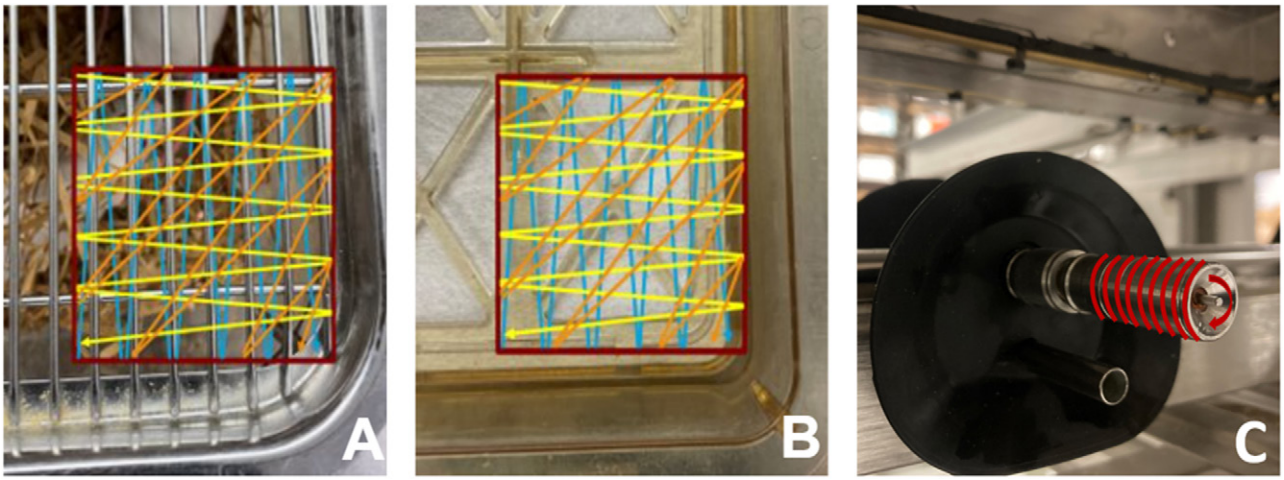

Figure 1. Swab collection pattern for each accessory. (A, B) Consistent 4- × 4-in. area at near right (when facing cage on individually ventilated caging rack) was swabbed on both the outside of wire bar lid and inside of filter top. Swabbing was performed in 3 directions with 10 passes each: vertically, horizontally, and diagonally as shown. The swab was rotated between fingers throughout swabbing to increase surface area contact. (C) The swab was passed 10 times around circumference of automated watering valve, with final swab being of front face portion. The swab was rotated between gloved fingers throughout swabbing.

and the cage was removed from the rack. Once a swab sample was taken, the ATP luminometer (SystemSure Plus; Hygiena, Camarillo, CA) was used per manufacturer recommendations to determine the amount of ATP present in the sample in RLUs.

Time and cost-savings analysis. We used our institution's average daily mouse cage census of 22,029 cages for all calculations. We compared a 2-wk cage component change to a 3-mo cage component change based on the findings of this study (see Results section). This analysis focused only on personnel labor time and cost savings, as labor was the primary variable that was affected by extending sanitation frequency to 3 mo. The amount of detergent, energy, and water costs used to run the rack washer and autoclave were not included because they are difficult to quantify. At our institution, utility costs are considered institutional costs, rather than part of the operational budget of the animal resources program, so we did not include utility costs in our analysis. Finally, because the tunnel washers in our facilities are idle throughout the work day when not actively washing equipment, we could not easily measure the energy savings associated with reducing the number of components processed through the tunnel washer. Another consideration is that cage components can be damaged or degraded over time with repeated washing and autoclaving, so decreasing the frequency of washing likely increases the lifespan of the equipment; however, this is also difficult to quantify and was not included in our analysis.

The time savings during cage change were analyzed by performing a time-in-motion study, timing an experienced animal care technician performing typical cage change duties on 2 single-sided racks. On one rack, a full cage change was performed, including cage bottoms, wire bar lids, filter tops, and automated water valves. On the second rack, only cage bottoms were changed, as would occur if the sanitation frequency of caging components was extended. The technician was experienced at performing both full cage changes and bottom-only cage changes, because our procedure at the time of the study was to change bottoms every 2 wk and to change components monthly. Both of the racks held 63 cages, and each rack housed 3 breeding cages with litters present. The 2 racks were changed on different days based on the cage change schedule, so the order of change was not randomized for this study. The time taken to set up and wheel in supplies, perform the actual cage change, and wheel dirty supplies to cage wash were measured. The total amount of time required for each scenario was divided by 63 to achieve an estimated time to change a single cage. Once the estimated time to perform a standard cage change and a bottoms-only cage change was established on a per-cage basis, yearly time taken to perform standard cage change and extended cage change of cage components was calculated. Standard cage change involved 26 standard cage changes for the year, whereas the extended cage component cage change involved 4 standard cage changes and 22 bottoms-only cage changes for the year. These numbers were then multiplied by our average daily cage census of 22,029 cages to achieve total amount of technician time per year spent performing standard cage change as compared with total amount of technician time per year spent performing cage change with extended sanitation interval of cage components. Cost savings were then calculated by multiplying the time savings by the average hourly cost of employing an animal care technician, including hourly wage plus fringe benefits, because this represents the true cost to the program.

The time savings during cage wash procedures was also analyzed by performing a time-in-motion study, in which an experienced cage wash technician was timed while loading a set number (2,000 units each) of wire bars lids and filter tops onto the tunnel washer, unloading the cage components from the tunnel washer, stacking the supplies onto rolling carts, and rolling them into and out of the autoclave. The amount of time taken to process cage components through the cycle of the tunnel washer was not included in the time-savings analysis, because cage wash technicians can perform other duties during that time. The automated watering valves were excluded from this portion of the analysis, because they are processed through cage wash in a bulk container, resulting in a negligible labor time for that activity. The amount of time to process a single component unit (1 wire bar lid + 1 filter top) through cage wash was calculated and then multiplied by the number of times each cage component unit would be processed per year (for standard cage change, this would be 26 times; for extended cage component sanitation interval, this would be 4 times per year). Time savings per cage per year was then multiplied by our average census to achieve a total amount of cage wash technician time saved per year. Cost savings were then calculated by multiplying

the technician time savings by the average hourly cost of employing a cage wash technician, including hourly wage plus fringe benefits.

Statistical analysis. Data were recorded into spreadsheets for recordkeeping (Excel; Microsoft, Redmond, WA). All analyses were performed in R-citation (R Foundation for Statistical Computing, Vienna, Austria). The amount of ATP (measured as RLU) between day 14 and every other time point was compared using paired wilcoxon signed-rank tests with a Bonferroni correction. This analysis was selected because the data distribution was not normal, and the sample size for the automated watering valve was lower at the 4-, 5-, and 6-mo time points. Data are expressed as mean ± SD, and differences were considered significant when $P < 0.004$, based on the Bonferroni correction. Statistical analysis was not performed for the time- and cost-savings study due to the large difference in time and cost, and the fact that this study was intended to be a practical assessment to highlight cost advantages for animal care programs considering the change to extended sanitation frequency of cage components.

Results

ATP luminometry. The ATP luminometry data demonstrated no significant difference in RLUs between 14 d and any other time point across all cage component groups: wire bar lids, filter tops, and automatic watering valves. For wire bar lids (Figure 2A), no significant differences in RLUs were detected when comparing the 14-d interval (1,207 ± 856 RLUs) with intervals of 28 d (1,442 ± 1,127 RLUs, $P = 0.046$), 56 d (1,253 ± 662 RLUs, $P = 0.623$), or 84 d (1,174 ± 824, $P = 0.789$). For filter tops (Figure 2B), no significant difference in RLUs were detected when comparing the 14-d interval (585 ± 575 RLUs) with intervals of 28 d (786 ± 1,115 RLUs, $P = 0.796$), 56 d (413 ± 360 RLUs, $P = 0.114$), or 84 d (369 ± 275 RLUs, $P = 0.025$). For automatic watering valves (Figure 2C), no significant difference in RLUs were detected when comparing the 14-d interval (1,057 ± 866 RLUs) with intervals of 28 d (1,313 ± 1,136 RLUs, $P = 0.136$), 56 d (1,015 ± 622 RLUs, $P = 0.546$), 84 d (980 ± 614 RLUs, $P = 0.684$), 112 d (898 ± 417 RLUs, $P = 0.816$), 140 d (1,033 ± 864 RLUs, $P = 0.632$), or 168 d (1,009 ± 568 RLUs, $P = 0.562$).

Time- and cost-savings analysis. For the time- and cost-savings analysis, personnel time per cage for a standard cage change, including setup and cleanup, was 1.8 min, and time per cage for bottoms-only change was 1.1 min. This resulted in 17,467 h spent yearly changing cages with the standard 2-wk cage change as compared with 11,653 h spent yearly changing cages with the extended sanitation frequency of cage components. Switching to a sanitation frequency of caging components to once every 3 mo resulted in savings during cage change of 5,816 h or $203,316 annually for our average daily census of 22,029 (Table 1). For every 10,000 cages, this equates to savings of 2,640 h saved or $92,295 annually.

For the cage wash analysis, time to process each unit of cage components (one wire bar + one filter top) was 0.15 min. Therefore, total time spent by cage wash technicians to process cage components using the standard 2-wk cage change would be 1,384 h yearly, and using the extended sanitation frequency of cage components would be 212 h yearly. Switching from a standard cage change to extended sanitation frequency of cage components results in a time savings of 1,171 h, or $37,308 annually for our census of 22,029 (Table 1). For every 10,000 cages, this equates to annual savings of 532 h saved, or $16,936 annually.

Including both the time saved during cage change and during cage wash, switching to an extended interval of sanitation of cage components to once every 3 mo would save a total of 6,987 technician hours annually, for a total cost savings of $240,623 for our census of 22,029 cages (Table 1). For every 10,000 cages, this equates to a total annual savings of 3,172 h and $109,230.

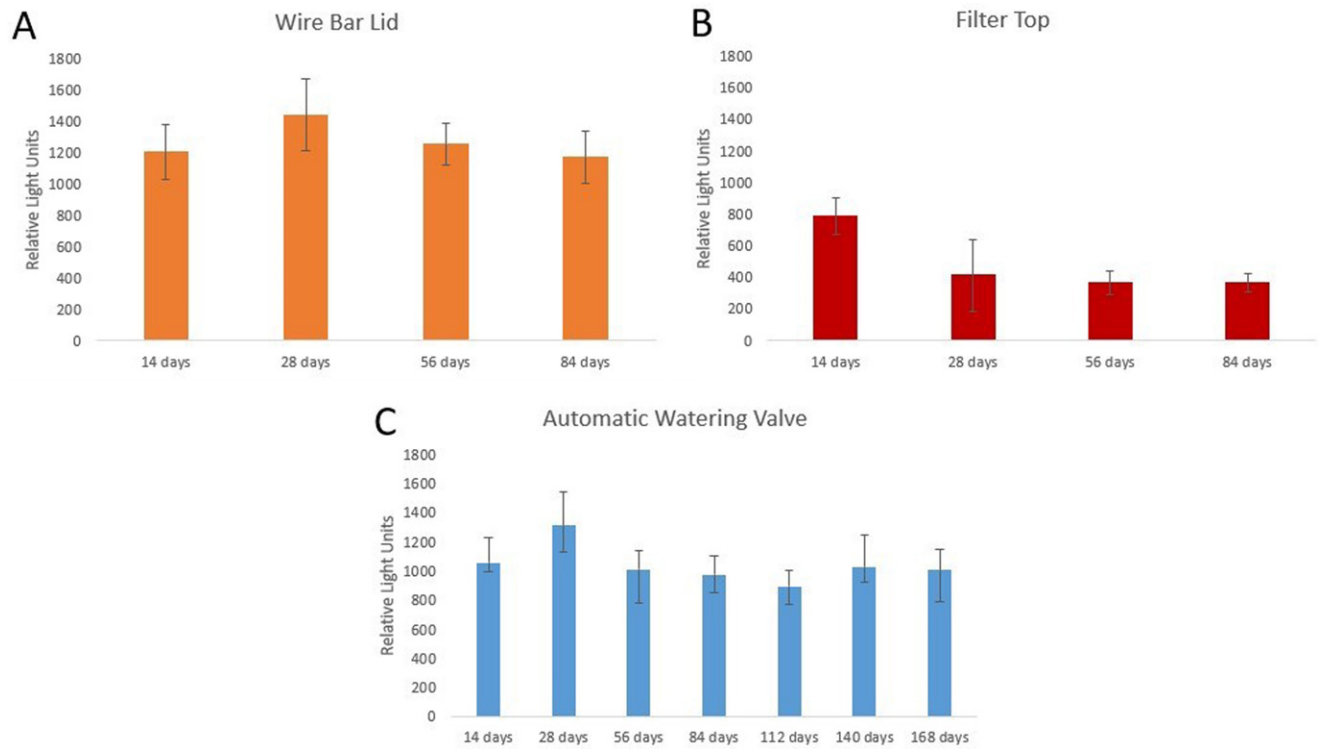

Figure 2. Mean ATP luminometer testing results in relative light units over time. No statistically significant differences were seen between any time point compared with baseline (14 d) across all cage component groups: wire bar lids (A), filter tops (B), and automatic watering valves (C).

Table 1. Summary of time and cost savings associated with using a 3-mo frequency for sanitation of cage components

Category	2-wk sanitation of cage components	3-mo sanitation of cage components	Difference (savings)
Annual time for cage change (h)	17,469	11,653	5,816
Annual labor expense for cage change ($)	610,715	407,399	203,316
Annual time for accessories in cage wash (h)	1,384	213	1,171
Annual labor expense for accessories in cage wash ($)	43,909	6,601	37,308
Annual total time saved (h)			6,987
Annual total labor expense saved ($)			240,623

Time savings were calculated based on time-in-motion studies of cage change and cage wash of accessories, assuming a mouse census of 22,029 cages. Cost savings were calculated based on the average hourly cost of employing animal care and cage wash technicians.

Discussion

Multiple studies have validated an extended sanitation frequency for rodent cage components (wire bar lids and filter tops)[2,3,13–15]; however, none of these studies have exactly duplicated the caging type, air changes per hour, bedding type, husbandry practices, and sanitation practices of our institution. Furthermore, no published reports have examined the extended sanitation frequency of automatic watering valves. We therefore performed the current study, which showed that wire bar lid and filter top sanitation could be extended to every 84 d (approximately 3 mo), and automatic watering valve sanitation could be extended to every 168 d (approximately 6 mo). In addition, our time-in-motion studies revealed that switching to a 3-mo sanitation interval of components would save approximately 7,000 h of technician time at our institution, which equates to approximately $240,000 in labor costs.

In this study, we used ATP luminometry to evaluate soiling of cage components. We chose this method over microbiologic quantification of colony forming units with RODAC plates due to the ability of ATP luminometry to detect pure cells and organic debris with a strong degree of linear predictability[16]; in contrast, RODAC plates detect only live bacteria that can be cultured readily. When assessing the level of soiling of caging components, we opted to use a method that measured all soiling due to organic debris compared with quantifying bacterial colonies to capture potential contamination of nonculturable bacteria, slow-growing bacteria, nonbacterial organisms such as fungal and viral contamination, and accumulation of waste in general. In addition, ATP luminometry is a simple and time-efficient method of quantifying organic material,[7] making it ideal for rapid analysis of the cage microenvironment.

The 2-wk ATP luminometry results were used as the baseline for comparison against all other time points. Because we found no significant differences in RLUs between the 2-wk time point and any other time point, we conclude that the degree of soiling of cage components (wire bars lids and filter tops) was not impacted by extending the frequency of sanitation to 3 mo, and that the degree of soiling of automatic watering valves was not impacted by extending the frequency of sanitation to every 6 mo. Despite the fact that automatic watering valves come into direct contact with the mice, the manufacturer does not explicitly recommend a specific sanitation interval. Furthermore, some automatic watering valves are permanently attached to the ventilated rack and thus are not easily sanitizable without removing the entire rack from the room. Our current study demonstrated that automatic watering valves, when wiped down externally once every 2 wk with disinfecting wipes, prevents an inappropriate amount of organic debris from accumulating on the valves. Not surprisingly, the RLU values of the filter tops were much lower than those of the wire bar lids and automatic watering valves, likely because of the fact that the mice typically do not come into direct contact with the filter tops.

Replacement of cage components could affect behavior and aggression in group-housed mice. Several studies have shown that the transfer of nesting material to the new cage at the time of cage change decreases aggression in group-housed mice[9,17]; however, the transfer of dirty bedding may increase aggression.[18] This phenomenon is thought to be because of the fact that urine in soiled bedding contains aggression-eliciting odors, while nesting material is relatively free of urine and feces and may contain hormones from the body that have been shown to inhibit aggression.[9,17] Cage components such as automated watering valves, wire bar lids, and filter tops are not likely to contain large amounts of urine or feces but may retain body odors, so extending the sanitation interval of cage components could reduce aggression in mice; however, this possibility requires further investigation.

Based on our findings that cage component sanitation can be extended to at least 3 mo, we conducted a time and cost savings by using time-in-motion studies to determine the savings that would be accrued by switching to an extended sanitation interval of cage components. Our analysis indicated that, for every 10,000 mouse cages, yearly technician time saved was approximately 3,000 h, or $10,000. This is the equivalent to the yearly working hours of more than one full-time employee per every 10,000 cages. Given difficulties in staff retention and hiring, switching to an extended sanitation interval of cage components could alleviate the burden of understaffing, and prevent employees from experiencing burnout due to unmanageable workloads. Although not examined in our analysis, some water and energy saving benefits are also likely to accrue in association with extending the sanitation interval of cage components; this would likely contribute to both cost savings and a positive environmental impact. Finally, reducing the number of times that components are processed through the tunnel washer and autoclave will likely extend their lifespan, which undoubtedly incurs cost savings. Cost and time savings will vary from program to program, depending on program-specific procedures that are used to perform cage change– and cage wash–related tasks. Therefore, institutions should perform their own time and cost analyses to determine the true savings for their specific program.

Although numerous publications support an extended sanitation interval for rodent cage components, AAALAC still requires each institution to verify environmental conditions as part of an internal performance standard for IVC cage change intervals longer than 2 wk. However, the many publications published that validate an extended sanitation frequency for cage components cover a wide range of experimental and housing variables that apply to many institutions (Table 2). With these numerous concordant publications available as references,

Table 2. Summary of published studies examining extended sanitation interval of rodent cage components on IVC racks

Reference	Species (sex)	Cage density (mice/cage)	Validation method	Autoclaved caging? (Y/N)	Bedding type	IVC type	Rack air changes per hour	Maximum sanitation frequency
3	Mouse (M and F)	4–5	Microbiology, ammonia levels	N	Paper-based	Thoren	30–60	42 d (wire bar and filter top)
2	Mouse and rat (sex of sentinels not stated)	3 (mice), 2 (rats)	ATP luminometry	Y	Hardwood	Allentown	60	90 d (wire bar), 180 d (filter top)
13	Mouse and rat (F)	5 (mice), 2 (rats)	Microbiology, ATP luminometry	N	Corncob	Lab Products (mice), Thoren (rats)	Unknown	90 d (mouse wire bar), 120 d (mouse filter top), 150 d (rat wire bar and filter top)
15	Rat (M)	2	Microbiology, ammonia levels, animal weight, hematology/chemistry, microbiome	Y	Aspen chip	Allentown	40	84 d (cage lid, box feeder, enrichment tunnel)
Current study	Mouse (M and F)	4–5	ATP luminometry	Y	Corncob	Allentown	60	84 d (wire bar, filter top), 168 d (automated watering valves)

IVC, individually ventilated caging.

guidance documents and regulatory agencies should consider a more flexible approach to rodent cage component sanitation frequency recommendations in the future.

To conclude, this study confirmed previous findings[2,13–15] that wire bar lids and filter tops for IVC caging do not accumulate significantly more organic debris over a 3-mo period and accessory change can therefore be extended safely without replacement for at least that duration. In addition, this study is the first to demonstrate that automatic watering valves require cage wash processing and autoclaving only once every 6 mo if they are wiped down every 2 wk with a sanitizing wipe. Finally, this study demonstrated a significant time and cost savings associated with extending the sanitation frequency of cage components, which can help to decrease workload without compromising the cage microenvironment.

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References

1. **Allen KP, Csida T, Leming J, Murray K, Gauld SB, Thulin J.** 2012. Comparison of methods to control floor contamination in an animal research facility. Lab Anim (NY) **41**:282–288. https://doi.org/10.1038/laban1012-282.
2. **Allen KP, Csida TJ, Thulin JD.** 2021. Assessing accumulation of organic material on rodent cage accessories. J Am Assoc Lab Anim Sci **60**:281–288. https://doi.org/10.30802/AALAS-JAALAS-20-000087.
3. **Ball BL, Donovan KM, Clegg S, Sheets JT.** 2018. Evaluation of extended sanitation interval for cage top components in individually ventilated mouse cages. J Am Assoc Lab Anim Sci **57**:138–142.
4. **Compton SR, Homberger FR, MacArthur Clark J.** 2004. Microbiological monitoring in individually ventilated cage systems. Lab Anim (NY) **33**:36–41. https://doi.org/10.1038/laban1104-36.
5. **Council NR.** 2011. *Guide* for the care and use of laboratory animals, 8th ed. Washington (DC): The National Academies Press.
6. **Doerning CM, Thurston SE, Villano JS, Kaska CL, Vozheiko TD, Soleimanpour SA, Lofgren JL.** 2019. Assessment of mouse handling techniques during cage changing. J Am Assoc Lab Anim Sci **58**:767–773. https://doi.org/10.30802/AALAS-JAALAS-19-000015.
7. **Ednie DL, Wilson RP, Lang CM.** 1998. Comparison of two sanitation monitoring methods in an animal research facility. J Am Assoc Lab Anim Sci **37**:71–74.
8. **Lappalainen J, Loikkanen S, Havana M, Karp M.** 2000. Microbial testing methods for detection of residual cleaning agents and disinfectants–prevention of ATP bioluminescence measurement errors in the food industry. J Food Prot **63**:210–215. https://doi.org/10.4315/0362-028X-63.2.210.
9. **Lidster K, Owen K, Browne WJ, Prescott MJ.** 2019. Cage aggression in group-housed laboratory male mice: an international data crowdsourcing project. Sci Rep **9**:15211. https://doi.org/10.1038/s41598-019-51674-z.
10. **Mailhiot D, Ostdiek AM, Luchins KR, Bowers CJ, Theriault BR, Langan GP.** 2020. Comparing mouse health monitoring between soiled-bedding sentinel and exhaust air dust surveillance programs. J Am Assoc Lab Anim Sci **59**:58–66. https://doi.org/10.30802/AALAS-JAALAS-19-000061.
11. **Memarzadeh F, Harrison PC, Riskowski GL, Henze T.** 2004. Comparison of environment and mice in static and mechanically ventilated isolator cages with different air velocities and ventilation designs. Contemp Top Lab Anim Sci **43**:14–20.

12. **Nascimento EA, Poveda VB, Monteiro J.** 2021. Evaluation of different monitoring methods of surface cleanliness in operating rooms. Rev Bras Enferm **74**:e20201263. https://doi.org/10.1590/0034-7167-2020-1263.
13. **Schondelmeyer C, Dillehay D, Webb S, Huerkamp M, Mook D, Pullium J.** 2006. Investigation of appropriate sanitization frequency for rodent caging accessories: evidence supporting less-frequent cleaning. J Am Assoc Lab Anim Sci **45**:40–43.
14. **Taylor JL, Noel P, Mickelsen M.** 2019. Evaluation of a 16-week change cycle for ventilated mouse cages. J Am Assoc Lab Anim Sci **58**:443–449. https://doi.org/10.30802/AALAS-JAALAS-18-000070.
15. **Taylor JZ, Fong DL, Habenicht LM, Fink MK, Leszczynski JK, Frank DN, Kofonow JM, et al.** 2023. Effects of extended cage component sanitation interval on the microenvironment, health, and gastrointestinal microbiome of rats (*Rattus norvegicus*). J Am Assoc Lab Anim Sci **62**:212–221. https://doi.org/10.30802/AALAS-JAALAS-22-000113.
16. **Turner DE, Daugherity EK, Altier C, Maurer KJ.** 2010. Efficacy and limitations of an ATP-based monitoring system. J Am Assoc Lab Anim Sci **49**:190–195.
17. **Van Loo PLP, Kruitwagen CLJJ, Van Zutphen LFM, Koolhaas JM, Baumans V.** 2000. Modulation of aggression in male mice: influence of cage cleaning regime and scent marks. Anim Welf **9**:281–295. https://doi.org/10.1017/S0962728600022752.
18. **Van Loo PLP, Van Zutphen L, Baumans V.** 2003. Male management: coping with aggression problems in male laboratory mice. Lab Anim **37**:300–313. https://doi.org/10.1258/002367703322389870.
19. **Vilar MJ, Rodríguez-Otero JL, Diéguez FJ, Sanjuán ML, Yus E.** 2008. Application of ATP bioluminescence for evaluation of surface cleanliness of milking equipment. Int J Food Microbiol **125**:357–361. https://doi.org/10.1016/j.ijfoodmicro.2008.04.024.