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Assessment of Mouse Handling Techniques During Cage Changing

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Mouse handling during cage changing and health evaluation has traditionally been performed by using forceps. This method was adopted as a biosecurity measure but can adversely affect employee ergonomics and rodent behavior. In this study, we evaluated alternative methods of rodent handling and their potential implications for efficiency, biosecurity, and animal welfare. Study groups included plastic cups, gloved hands, 2 methods of tunnel handling, and forceps. Evaluations included speed of cage change, ATP-based assessment of sanitization, and retrospective analysis of colony health and breeding data. The time to change 14 cages was significantly faster at each time point for the gloved hands and forceps groups as compared with the other methods. Overall speed did not increase significantly with each subsequent study week for any group. ATP levels after sanitization with hydrogen peroxide–peracetic acid mixture differed significantly between gloves and forceps. When ATP level was evaluated on a per-cm^2 basis, no significant difference between gloves and forceps was detected. Although tunnel and cup handling both increased the time for cage-changing, the tunnel served as both an indirect handling method and a shelter when left within the cage. Retrospective analysis revealed that breeding performance and colony health were similar among groups. Although efficiency is a concern for large-scale implementation of novel handling methods, the tunnel method may prove beneficial for sensitive strains or studies requiring indirect handling. In addition, using gloved hands to directly handle mice during cage changing is efficient and avoids the ergonomic strain associated with forceps. Precautions should be taken when handling mice with gloves, given that the increased contact area carries an increased load of organic debris. Changing gloves between rack sides or before proceeding to the animals belonging to a different investigator minimizes the potential for cross-contamination.

Abbreviation: RLU, relative light units

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The use of forceps for mouse handling is a widely accepted husbandry practice in the animal research community. This handling method has traditionally been implemented according to biosecurity recommendations to decrease the potential for pathogen exposure between animals housed in biocontainment and to prevent exposure of sensitive animals (for example, immunocompromised, SPF) to unwanted pathogens and opportunistic organisms.[5] This method is performed by using padded-tip forceps to gently grasp mice at the base of the tail or the loose skin around the nape of the neck.[9] Given the number of mice handled daily, the simple grasping motion is recognized as a highly repetitive action for animal care personnel performing cage changes.[10,11] Combined with ergonomic strain, this repetitive action creates a high risk for musculoskeletal injury to diverse body areas in addition to the hand and wrist: the elbow, shoulders, neck, and back can all be affected.[10] Recommendations to minimize musculoskeletal strain include the use of ergonomically designed forceps, antifatigue mats, and alternation of hands and reducing the proximity to the cage changing station to accommodate a caretaker's reach.

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Alternative methods of mouse handling have previously been explored and include direct handling with gloved hands and indirect handling by using various equipment, including PVC or polycarbonate tubes, plastic scoops, and enrichment devices already present within the cage. When implementing alternative mouse handling methods, considerations should extend beyond ergonomics and biosecurity. The influence of routine handling on animal welfare and behavior is increasingly being explored, and several studies have emerged that encourage the research community to refine standard handling practices to optimize animal welfare and promote sound science. In one study, picking up mice by the base of the tail resulted in higher anxiety and handler-associated aversion than did using a tunnel or an open hand.[7] Another study compared mice that were picked up by the tail with animals that caretakers cupped in their hands; the mice handled with cupped hands showed reduced anxiety and blood glucose and increased glucose tolerance.[2] Additional studies suggest that handling methods have the potential to act as a research confounder. For example, gentle handling was found to mitigate depressive-like behavior in neuropsychiatric models, whereas mice handled aggressively displayed more severe depressive-like behaviors.[14] Another study showed that handling mice by the tail can significantly influence behavioral testing by exacerbating neophobia in a novel testing environment; using a tunnel for nonaversive handling resulted in more robust investigatory behavior.[4] These cited studies demonstrate that even the methods used for tasks as apparently straightforward

as rodent handling can have significant implications on animal wellbeing and research outcomes.

Our current study had 3 goals. The first goal was to provide animal care personnel with alternatives to using forceps for mouse handling during cage changing. As for any programmatic change, ensuring that a new handling process does not create additional burdens of labor or time is essential. We evaluated the standard forceps-handling method, direct handling with gloved hands, and the use of tunnels and plastic cups, to assess the feasibility and efficiency of these methods during the cage-changing process. Given the behavioral results of similar studies, which showed an acclimation response to novel handling techniques,[2,4,7] we hypothesized that both animals and the handler would adapt to the alternative handling methods and that this adaptation would be reflected as an increased speed of cage changing as the weeks progressed.

Our second goal was to assess the biosecurity implications of these handling methods, with a particular focus on sanitization efficacy as an indicator of the potential for disease transmission. We used ATP monitoring, similar to previous sanitization studies in animal research facilities.[1,6,17,19] We hypothesized that our existing microisolation technique of using a disinfectant dip would significantly remove organic contamination, reflected as a decrease in ATP from before to after dipping. Third, to determine any effects of handling techniques on reproductive performance and the caretaker's ability to perform health examinations, we retrospectively analyzed breeding and animal health records generated during the study period. We hypothesized that handling techniques would not affect breeding performance or the caretaker's ability to perform health examination.

Materials and Methods

Animals. Our facility's animal care and use program is AAALAC-accredited. All procedures and housing were compliant with the *Guide for the Care and Use of Laboratory Animals*, 8th edition[8] and were approved by the University of Michigan's IACUC. The study population consisted of 70 cages of mice of varied strains and ranging from neonatal pups to 6-mo-old animals (n = 242 adults) in a preexisting breeding colony. Mice were free of mouse hepatitis virus, minute virus of mice, mouse parvovirus, enzootic diarrhea of infant mice virus, ectromelia virus, Sendai virus, pneumonia virus of mice, Theiler murine encephalomyelitis virus, reovirus type 3, lymphocytic choriomeningitis virus, mouse adenovirus, polyomavirus, *Mycoplasma pulmonis*, fur mites, and pinworms, according to results from the surveillance program. Mice were housed either singly or in groups of as many as 5 adult mice per IVC (P/NV IVC, Allentown Caging, Allentown, NJ). Cages containing litters were limited to 2 adult mice, to comply with university guidelines. All cages contained 1/4-in., irradiated corncob bedding (Bed-o-Cobs, The Andersons, Maumee, OH) and nesting material (Enviropak, WF Fisher and Son, Branchburg, NJ). A subset of mouse cages (n = 14) received a clear polycarbonate tunnel (9.84 cm × 5.08 cm; Mouse Tunnels Certified, Bio-Serv, Flemington, NJ) that remained within the cage as part of the experimental design. Mice had unrestricted access to a commercial rodent diet (PicoLab Laboratory Rodent Diet 5L0D or PicoLab Laboratory Rodent Diet 5008, PMI Nutrition International, St Louis, MO) and triple-filtered city water through an automated watering system. Animal rooms were maintained at 72 ± 2 °F (22.2 ± 1.1 °C) and 30% to 70% relative humidity and were on a 12:12-h light:dark cycle. Throughout the course of the study, no study animals received manipulation necessitating anesthesia or analgesia or experienced adverse events requiring veterinary intervention.

Study groups and handling techniques. Study cages were divided into 5 experimental groups according to the method of handling and transferring to a clean cage for routine cage changeout. Each group consisted of 14 cages, with a balanced distribution of breeding pair and single-sex cages (male or female). The groups were: home tunnel, mice transferred by using a tunnel from their home cage (7 cages of breeding pairs, 5 all-male cages, 2 all-female cages); novel tunnel, mice that were exposed to tunnel handling only during cage changing (7 cages with breeding pairs, 5 all-male cages, 2 all-female cages); cup, mice transferred by using a 60-mL polypropylene (bisphenol A–free) plastic food-service cup (ChoiceHD, Webstaurant Store Food Service Equipment and Supply, Lancaster, PA; 7 cages of breeding pairs, 4 all-male cages, 3 all-female cages); gloves, groups for which the caretaker used gloved hands to transfer mice by grasping the tail (7 cages with breeding pairs, 5 all-male cages, 2 all-female cages); and forceps, groups for which the caretaker used stainless steel rubber-tipped forceps (Surgical Design Millers Forge Stainless-Steel Specimen Forceps, Thermo Fisher Scientific, Waltham, MA; C-Flex laboratory tubing, Sigma-Aldrich, St Louis, MO) to transfer mice by grasping the tail (7 cages of breeding pairs, 5 all-male cages, 2 all-female cages). All procedures were performed within a laminar flow cage changing station (Phantom Animal Transfer Station, Allentown Caging). For cages with litters, the nest containing mouse pups was scooped in gloved hands and moved to the clean cage; according to our standard operating procedures, cages with new litters were not changed for at least 24 h after parturition unless at least 25% of the cage bedding material was wet. Once pups became relatively active (that is, at approximately 14 d of age), the handling technique for each experimental group was used.

Gloved hands and handling materials were disinfected between cages by using a 1:4 dilution of hydrogen peroxide–peracetic acid mixture (Spor-klenz RTU, STERIS Life Sciences, Mentor, OH). Mice in the home tunnel group were transferred in their existing tunnel to a clean cage, and a clean tunnel was provided in each new cage. For the novel tunnel group, 2 tunnels were alternated and were disinfected in a dip box (a standard mouse cage) containing disinfectant solution. Two cups were used also, alternating in disinfection in the dip box between cages. Cups were treated as disposable and discarded after each study group. For the gloves group, caretakers dipped their gloved hands into disinfectant between cages of mice and changed gloves between study groups. For the forceps group, 2 pairs of forceps were alternated and were placed in the dip box between cages. Forceps, tunnels, and disinfectant dip boxes were sanitized through cage wash at least weekly and more frequently as needed.

Assessment. To assess speed of cage changing by a single animal-caretaker, timing was performed on a per-cage basis by using a stopwatch (Fisherbrand Traceable Mini-Alarm Timer Stopwatch, Thermo Fisher Scientific); timing began when a cage was placed into the cage changing station and ended when the newly changed cage was removed from the station. Each of the 14 cages within an experimental handling group were changed consecutively. The order in which each group was changed was randomized at each cage-change interval. Timing assessments were performed at 4 separate cage-change sessions, with a minimum of 4 wk between timing assessments. Between these timing assessments, handling techniques for each group were continued.

An ATP-based detection system (novaLUM, Charm Sciences, Lawrence, MA) was used to assess the level of microbial and organic contamination (expressed as relative light units [RLU])

of surfaces at multiple time points throughout the cage change process. A single swab (PocketSwab Plus, Charm Sciences) was used to sample each group (that is, handling material at each time point). Only surfaces that came in direct contact with the mice and the cage microenvironment were sampled for each group: outside and inside of the tunnels and cups for the novel tunnel and cup groups; front and back of the padded tips of the forceps for the forceps group; and palms and front and back of each finger of both gloved hands for the gloves group. Sampling was not performed for the home tunnel group, because each tunnel was used only to transfer mice from that specific cage. Swabs were collected at baseline (that is, before coming into contact with mice or caging) and immediately after the set of 14 cage changes. In addition, a final swabbing was also performed for the novel tunnel, gloves, and forceps groups. Tunnels and forceps were sampled after a 2-min submersion in the dip box, according to vendor recommendations, and gloves were sampled after a final dip. ATP sampling data were collected for each group at 4 separate cage-change intervals.

In addition, the disinfectant solution in the dip box was sampled directly by briefly stirring a swab (WaterGiene, Charm Sciences) in the solution after each study group. The dip box and disinfectant liquid were confirmed as visibly clean prior to the start of each evaluation. The first evaluation involved disinfectant solution that was not changed between groups (group order listed in Table 1), whereas the second entailed rinsing the dip box with tap water and filling it with fresh disinfectant prior to the next group of cages.

Animal health reports generated by veterinary and husbandry technicians during daily cage observations were collected throughout the study for comparison between groups. Breeding data regarding litter size were obtained from the colony manager for comparison between groups. Pups from litters were genotyped and removed before weaning at the discretion of researchers; therefore, the number of pups weaned was excluded from analysis.

Statistical analysis. Two-way ANOVA with Tukey multiple comparisons test was used to compare speed of cage change, by using handling method and week as grouping factors. The approximate surface area swabbed for testing was calculated for each handling instrument, and mean ATP level was divided by surface area to estimate ATP (RLU) per cm^2. For both overall and per surface area ATP measurement, one-way nonparametric ANOVA (Kruskal–Wallis test) with Dunn multiple comparisons test was used to compare mean ATP levels between handling instruments. Breeding data were analyzed by using repeated-measures one-way ANOVA. A P value of 0.05 or less was considered significant for all analyses.

Results

Handling assessment. During session 1, statistical differences in cage-change speed were found for home tunnel compared with gloves ($P = 0.0416$), novel tunnel compared with gloves ($P = 0.0058$), novel tunnel compared with forceps ($P = 0.0452$), and cup compared with gloves ($P = 0.0382$). In all significant comparisons, gloves or forceps were found to be faster than other methods.

During session 2, home tunnel was significantly ($P = 0.0096$) faster than novel tunnel and gloves were significantly ($P = 0.0058$) faster than home tunnel. Novel tunnel was significantly slower ($P < 0.0001$) than gloves, cup, and forceps and was the slowest method overall.

During session 3, both gloves ($P < 0.0001$) and forceps ($P = 0.0001$) were faster than home tunnel. Similar significant differences were found for novel tunnel compared with gloves or forceps ($P < 0.0001$ for both) and cup compared with gloves ($P < 0.0001$) and forceps ($P = 0.0007$), with gloves and forceps usage leading to faster cage changes than novel tunnel or cup.

During session 4, the home tunnel group had a significantly slower cage change rate than all other groups ($P < 0.0001$ for novel tunnel, gloves, forceps; $P = 0.0123$ for cup). The remaining difference during session 4 was between cup and gloves, with gloves significantly ($P = 0.0096$) faster.

Contrary to our initial hypothesis, overall cage-change time during the last cage-change session was not faster than during the first for any handling method. No significant difference in speed was found when comparing session 1 with session 4 for the novel tunnel, cup, gloves, or forceps groups. Interestingly, the home tunnel group showed a difference between sessions 1 and 4, with a significant ($P < 0.0001$) slower cage-change speed at session 4 (Figure 1).

Sanitization assessment. No significant differences were detected when the baseline mean ATP levels from the surfaces of the novel tunnel, cup, gloves, and forceps groups were compared. The second sampling, after each group of 14 cage changes, showed no significant differences between groups (Figure 2). The final sampling collected from the novel tunnel, gloves, and forceps groups after dipping showed a variable decrease in ATP level from before to after sanitization across groups. A difference was detected between the gloves and forceps groups, with forceps having a significantly ($P = 0.0051$; Figure 2) lower RLU than did gloves after sanitization sampling.

A second test was performed to estimate the ATP level (that is, number of RLU) per sampled cm^2 of each handling instrument. The calculated surface area of each instrument was as follows: novel tunnel, 650 cm^2; cup, 1690 cm^2; gloves, 200 cm^2; forceps, 10 cm^2. No significant differences were detected at baseline or after the 14 cage changes (Figure 3). At the final sampling point after sanitization, a difference was detected between the novel tunnel and gloves groups, with novel tunnels having significantly ($P = 0.0098$; Figure 3) lower RLU per cm^2 than gloves.

Table 1 shows the ATP levels of the disinfectant solution. The first evaluation, in which the solution was not changed between groups, revealed a steady increase in ATP after each successive group of cage changes. The liquid appeared cloudy and contained visible cage debris after each group. In contrast, the second evaluation revealed that the ATP levels remained comparable between groups when disinfectant was replaced after each group. The ATP levels remained elevated in the cup and novel tunnel groups despite changing disinfectant solution.

Animal health and breeding assessment. Analyses of animal health reports ($n = 8$, Table 2) generated throughout the study did not reveal an increase in the frequency of health conditions between groups. No significant differences were found between home tunnel, novel tunnel, cup, or gloves handling when comparing the average number of pups per litter born during the study (Figure 4). Health and breeding data for cages in the forceps handling group were unavailable for retrospective analysis.

Discussion

Strong evidence supports that routine handling methods have effects on mouse welfare and research outcomes.[2-4,7,15] Indirect handling methods, such as the use of a metal spatula, have proven beneficial for fragile mouse models, such as the osteogenesis imperfecta (*oim*) strain.[18] The 2 forms of tunnel handling evaluated in the current study have been described elsewhere.[8] In addition, the implications for workflow efficiency,

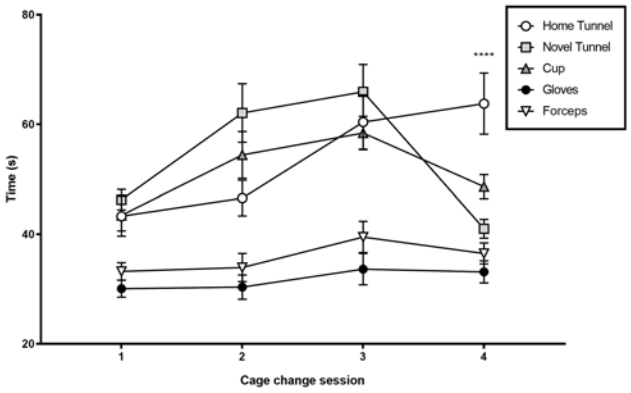

Figure 1. Time (s; mean ± SEM [bar]) to change each cage. Timing was evaluated for each handling group on 4 cage changing sessions (n = 14 cages per handling group). In the home tunnel group, the time to change 14 cages was significantly ($P < 0.0001$) slower during session 4 compared with session 1. Cage change speed did not differ between sessions 1 and 4 for any of the remaining groups.

training animal care staff, and sanitization must be considered when assessing novel husbandry practices in a rodent facility.

Contrary to our hypothesis, the speed of cage changing did not increase consistently for any handling method. This effect is likely due to both animal- and personnel-associated factors. In previous studies that focused on behavioral outcomes of handling, mice were maintained in very specific population and housing configurations, including sex, strain, and age-matched pairs or groups. In contrast, our study population consisted of a transgenic mouse breeding colony with varying strains, ages, sexes, and cage densities. We attempted to balance the distribution of single-sex and breeding cages across each handling group; however, cage turnover did occur during the course of the study. For example, a breeding cage with a litter of 8 pups during the first study session may have had all pups weaned and removed from the study at a later session. Although the turnover in cage density is a considerable source of intercage variation, it allows for a more practical assessment of the handling methods during the cage-change process, given that animal care personnel may work with diverse populations of mice within a given room, often within the same housing rack. In addition, handling sessions occurred on a more consistent basis in previous studies compared with the current study.[3,4,7,15] The acclimation effect on the animals may have been delayed or negated in our study, because cage-change sessions occurred once every 2 wk over the course of the study. With more frequent handling sessions, which often occurs when laboratory personnel handle mice for experimental purposes, a more significant acclimation curve may result.

The trend of increasing time to change 14 cages during the first 3 cage-changing sessions may reflect that the multiple methods of handling were still in a learning or acclimation phase for the technician. Performing different handling methods one after another could have been a challenge in the caretaker's mastery of each and all techniques. However, because the caretaker was able to develop a changing rhythm by using one designated method for each set of 14 cages, this explanation is rather unlikely. Except for the home tunnel, the time for all methods decreased during the 4th cage change session. A plausible explanation for this may be the acclimation of mice to the tunnel left within the cage. As the mice acclimate to the tunnel, it serves a dual purpose as a shelter as well as a handling device. The propensity of mice to nest within the tunnel may lengthen the amount of time it takes to perform health checks or cage changing, but despite this finding, leaving the tunnel in the mouse cage serves as a source of enrichment and shelter, which has been reported to contribute positively to mouse welfare.[3] Extending the duration of the study to evaluate subsequent generations of mice may provide more information regarding learning and acclimation to various handling methods.

The forceps and gloves groups had faster cage-change times than the remaining groups at each time point. These findings were likely due to using forceps or a gloved hand to handle mice as common techniques for mouse handling during cage changing and health examination, respectively, in our institution, as described in our current standard operating procedures. The study caretaker indicated that the use of gloved hands to directly handle animals was more intuitive, especially not requiring the use of any instrument. Although the forceps method produced a similar speed of cage-change, it predisposes employees to develop musculoskeletal strain when handling a high volume of mice.[10] Meanwhile, the advantages of using tunnels over disposable cups include the ability to sanitize and reuse the tunnels. In addition, the use of clear polycarbonate materials, as previously recommended,[7] allows for visualization of animals within the tunnel both cage-side and during cage-change transfer. Although we initially assumed that the technique of scooping animals with a cup would be easier than using a tunnel, mice were able to grasp the cup rim. Although this factor did not cause a significant time difference in our study, it may be considered a potential time cost to animal handlers.

In addition, we evaluated the time and material cost of using a tunnel handling method. The average time in our study to change 14 cages with gloved hands and with a tunnel were 7.5 and 12.5 min, respectively. Extrapolating this difference to an average of 200 cage changes a technician may perform per day, the additional 5 min to change 14 cages increases to a total of 70 min. Furthermore, the capital investment required to acquire clear polycarbonate tunnels for cage-changing is considerable, because each tunnel used in this study cost $3.70. Tubes of similar composition at lower price points may be acquired from other vendors. However, cost factors should not outweigh the benefit of gentle handling on animal welfare and research variability.[3,4,7]

The sanitization assessment revealed that all handling methods resulted in ATP levels that did not vary significantly after 14 cage changes. After sanitization—that is, after the disinfectant dip—the ATP levels for the gloves group did not decrease, in contrast to that of the forceps groups. This difference was likely due to the contact time, because the forceps were submerged in the dip box for 2 min, whereas gloved hands were merely dipped according to standard microisolation technique. Although the process for sanitization was similar to that of the forceps, the ATP levels for the tunnels did not differ significantly before and after sanitization. This result may have been because organic debris was rinsed off the smooth, polycarbonate surface of the tunnel more easily in the disinfectant, as compared with the tips of the forceps, which were coated in rubber. The high ATP levels before sanitation for the cup group are likely because the material was food-grade, arriving in sealed but not sterilized packaging. The process of running the tunnels and forceps through the facility cage wash prior to first use may explain why these groups had lower ATP levels before sanitation.

The same data were also evaluated according to the surface area of the transfer devices because the direct-contact surface varies greatly between each handling instruments. This analysis revealed that the forceps had the highest ATP level

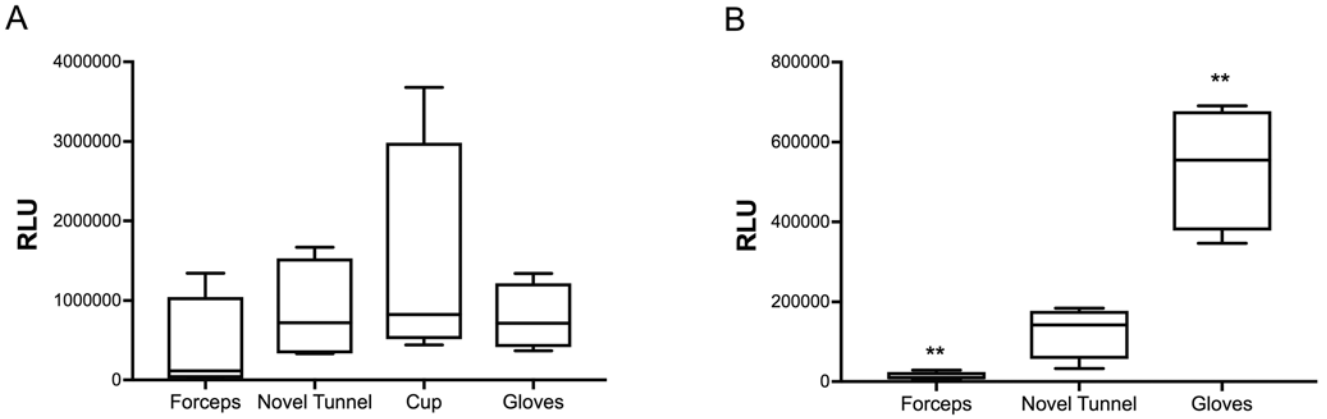

Figure 2. ATP levels according to each handling instrument. No significant difference was detected before sanitization. (A) After 14 cage changes, presanitation ATP levels did not differ significantly between any instruments, although the forceps showed high variability. (B) The forceps and gloves groups differed significantly ($P = 0.0051$) after sanitization by using the dip box, with forceps having a lower RLU level compared with gloves.

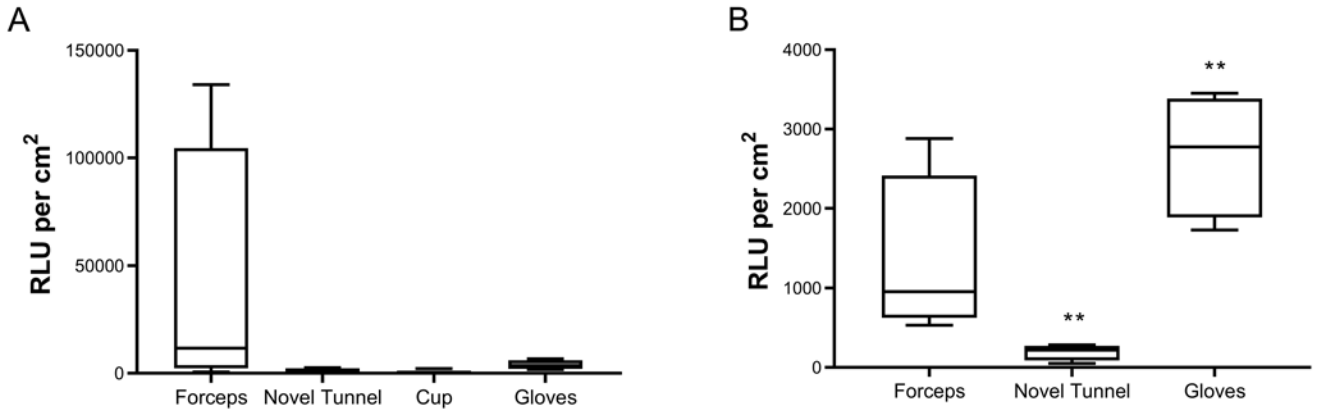

Figure 3. ATP levels (counts per cm^2) for each handling instrument. (A) After 14 cage changes, presanitation ATP levels did not differ significantly between any instruments, although the forceps showed high variability. (B) The novel tunnel and gloves groups differed significantly ($P = 0.0098$) after sanitization in the dip box, with novel tunnels having fewer RLU per cm^2 than gloves.

Table 1. ATP levels (no. of RLU) of disinfectant solutions.

Treatment	Group	no. of RLU after 14 cage changes
Disinfectant	Home tunnel	235553
not changed	Novel tunnel	288304
after each group	Cup	419117
	gloves	409100
Disinfectant	Home tunnel	8851
changed	Novel tunnel	46801
after each group	Cup	53003
	gloves	651
	forceps	2142

ATP levels increased when the disinfectant solution was not changed after each group of cages ($n = 14$). In contrast, ATP levels were comparable among groups when the dip box was rinsed and filled with fresh disinfectant after each group. Note: the ATP level for the forceps group was evaluated during the second treatment phase only.

per cm^2 after cage-changing. The forceps ATP levels remained high after sanitization, indicating that simple immersion into disinfectant solution was not effective to remove debris from the rubber-coated tips. The overall high concentration of ATP level per cm^2 for the forceps was not surprising, because it had the smallest direct-contact surface area compared with tunnel and gloved hand. Gloves again displayed the highest ATP level after sanitization. We chose a hydrogen peroxide–peracetic acid mixture as our broad-spectrum disinfectant for this study. Care should be taken when selecting the disinfectant used during a cage-changing process, given that required contact time and efficacy against organic debris can vary greatly among commercial disinfectants. For example, the manufacturer of the disinfectant we used recommends removing obvious debris and organic material prior to immersing the items to be sanitized. The contact time recommended for germicidal efficacy with this product is 30 s, whereas the contact time for sanitization is recommended as 5 min. In addition, the choice of disinfectant should be evaluated for effects on direct handling of animals. When alternative handling methods are used, animals experience increases in contact time and surface area to disinfectants. Although no adverse effects of contact with the disinfectant were noted in study animals, chemical irritation or systemic toxicity may be apparent with different classes of disinfectant.

The ATP level does not distinguish between live and dead organic debris.[12,19] In addition, standard ATP level cutoffs do not exist for evaluating the efficacy of sanitization in an animal care institution, and acceptable limits are often specific to the institution.[6,19] Although this assay is a sensitive indicator of surface sanitization, it is not specific for the type of contami-

Table 2. Animal health reports generated during the study (n = 8).

Group	Cage	Report	Time of observation
Home tunnel	Breeding pair + litter	Delayed wean	Cageside
	Breeding pair	Male with ear lesion	Cageside
	Breeding pair + litter	Delayed wean	Cageside
Novel tunnel	Breeding pair	Male with ear lesion	Cageside
	Breeding pair	Female with nape lesion	At cage change
Cup	Adult males	Malocclusion	At cage change
	Breeding pair + litter	Female with nipple lesion	At cage change
gloves	Breeding pair + litter	Delayed wean	Cageside

Handling method did not appear to influence the ability to detect abnormalities at cage change. Home tunnel did not appear to inhibit the ability to conduct cageside health observations.

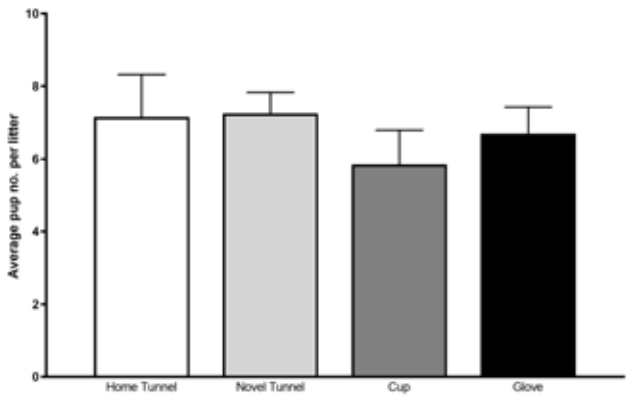

Figure 4. Number of pups born per litter (mean ± 1 SD [bar]) in each handling group throughout the study. The number of pups born was similar for all groups.

nation detected or for evaluating the true germicidal efficacy of the disinfectant product. The risk for transmission of live, potentially pathogenic organisms cannot be assessed directly by using this assay and requires further evaluation, such as through microbial culture or PCR testing.

We also used reproductive performance as an animal health indicator, and we did not find any significant differences in breeding performance over the course of the study. In addition, health records did not reveal an increased frequency of animals found sick or dead at cage change or during daily cage-side observations among handling groups, suggesting that the handling methods did not negatively affect the caretaker's ability to detect health conditions in individual mice during cage changing.

Given our results, our institution revised the existing husbandry practices to allow animal care personnel to use gloved hands as an alternative to forceps when handling mice during cage changeout. Although the sanitization results indicated that gloved hands had higher ATP levels than forceps and tunnel after sanitization, our risk assessment considered SPF-status contamination of our rodent colonies minimal because the prevalence rate of murine pathogens in our program has been considerably low as detected by our quarterly surveillance testing (25 reported contaminations from 2016 through early 2018 among approximately 200 mouse holding rooms). The use of gloved hands does not apply to husbandry practices in biocontainment areas and during disease outbreaks. In addition, the importance of adhering to institutional glove-changing guidelines was emphasized. These include changing gloves between investigators' colonies and between sides of a rack and sanitizing the disinfectant dip box through cage wash at least weekly. Forceps, sanitized at least weekly through cage wash, still remain in each mouse room, should personnel prefer to use them. The use of tunnels or cups was not implemented because of excessive labor and cost for a programmatic change. Although the use of devices for mouse transfers during cage changeout has previously been shown to have a positive effect on animal welfare and decreasing research variability,[3,4,7] further investigations on their use, such as pertaining to labor efficiency, may be warranted. Additional evidence supports the use of gloved hands as a refinement over forceps when considering factors other than reduction of anxiety-like behavior. Forceps-handled male mice have shown increased levels of aggression as compared with tunnel and gloved tail-handling, and consistent, gentle tail-handling has been shown to have a mitigating effect on depressive-like behavior as compared with aggressive or minimal handling.[13,14]

One limitation of our study is that we involved an animal caretaker who was experienced in rodent behavior and handling practices. Despite being naïve to the use of a tunnel and cup for mouse handling, we postulate that the relative overall experience contributed to how quickly novel handling methods were learned. A further area of investigation could include a timing comparison with a less-experienced animal handler. Other future directions include the performance of standardized behavioral assessments to determine whether anxiety differed among handling techniques. The health and breeding assessment from animals in the forceps group was not included, because the exact cage numbers and locations were not recorded at the time of the study, thus precluding the ability to identify the records retrospectively. Despite this oversight, the combined observations from the veterinary, husbandry, and research staff revealed no adverse events or unexpected morbidity or mortality within the colony throughout the course of the study, regardless of group. In addition, because forceps handling was standard practice prior to the initiation of this study, significant health detriment was not expected to occur. However, institutional practices may vary from liquid disinfectant to include sterilization of tools with a hot-bead sterilizer. For institutions that use this method of sterilization during the animal handling and cage change process, evaluation of health reports to may be of value to determine whether dermatitis or burn lesions are occurring as a result of the practice.

Refinement, as described by Russell and Burch,[16] refers to the minimization of animal suffering in the research setting with subsequent improvement in animal welfare. In a broader sense, this definition also means finding and implementing alternative ways to improve practices in routine care of animals. Although our current study led to a small change in husbandry practices, our strategy to evaluate the implications of new handling methods on sanitization, efficiency, and animal health forms the basis for large-scale assessments that can benefit animals and the personnel that provide their daily care.

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