Effectiveness of Various Floor Contamination Control Methods in Reducing Environmental Organic Load and Maintaining Colony Health in Rodent Facilities

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Floor contamination control practices in rodent housing facilities commonly include disposable shoe covers despite the lack of evidence for their usefulness in bioexclusion. Contamination control flooring mats are advertised as an economical and environmentally-responsible alternative to shoe covers, yet little is published regarding their efficacy in preventing the transfer of organic material and the introduction of infectious agents into facilities. We evaluated 4 floor contamination control strategies—shoe covers (ShCv), contamination control flooring (CCF), using both products concurrently (ShCv+CCF) compared with using neither—in preventing bacterial transfer and reducing organic load on facility floors and maintaining murine colony health status. According to PCR assay and culture analysis, ShCv provided the greatest reduction in bacterial numbers. Either ShCv, CCF, or ShCv+CCF significantly decreased ATP levels within the facility compared with those at facility entrances, with ShCv+CCF yielding the greatest reduction; however, even when neither ShCv nor CCF was used, intrafacility floor ATP levels were about half those at entrances. According to PCR analyses, no murine parasitic, viral, and bacterial pathogens excluded at the institution were detected in any floor, exhaust air dust, or sentinel samples at any time or location, regardless of the floor contamination control method in use. These findings show that floor contamination control methods help to reduce the organic load in rodent IVC facilities but do not enhance protection from environmental contamination due to murine pathogens.

Abbreviations: CCF, contamination control flooring; EAD, exhaust air dust; ShCv, shoe covers; RLU, relative light units

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The introduction of murine pathogens into an animal facility poses a risk to the health and wellbeing of laboratory animals. An outbreak of excluded murine pathogens could result in increased animal morbidity and mortality and significantly disrupt research operations and experimental outcomes. An adequate response to such an outbreak could cost the animal care and use program substantial time and resources due to diagnostic testing, animal quarantine, and decontamination. In addition, a breach in lab animal biosecurity could delay or preclude intermural research efforts. Therefore, preventing adventitious infections in laboratory mice is essential and necessary for animal health and wellbeing, successful research, and consistency with the 3Rs.¹⁶

Personnel can be an unknowing mechanical vector of murine pathogens.⁷ Conscientious facility design and standard operating procedures that direct the flow of foot traffic from noncontaminated areas into contaminated areas within a facility reduce the risk of inadvertently transmitting pathogens. The use of PPE, such as gloves, gowns, and shoe covers (ShCv), is another method of mitigating the potential mechanical vectorborne and fomite spread of pathogens by personnel,⁷

however the effectiveness of PPE for bioexclusion remains unsubstantiated.^{9,25} ShCv are used frequently in rodent facilities, but their widespread use has largely been based on historical practices rather than documented evidence of protecting against disease outbreaks.⁹ ShCv are expensive and detrimental to the environment and may result in ergonomic issues related to bending over and twisting during donning and frequent changing;⁹ for these reasons, the costs of using ShCv do not justify their use given their unsubstantiated benefit. Results from a study using contamination simulation powder in a rodent barrier facility indicated that ShCv had no effect on the spread of powder in the facility and that donning ShCv may be a source of contamination of gloves and therefore compromise bioexclusion; these findings led the authors to recommend the elimination of ShCv use in their facilities.⁹ Another study looked at reducing required PPE to water-resistant sleeves and gloves only and determined that a reduced level of PPE combined with microisolation technique can be used to maintain mice SPF for mouse norovirus and mouse hepatitis virus in an endemically infected colony in both static caging and IVC.³ In addition, the efficacy of ShCv has been considered in human hospitals, where the authors found that the strict use of ShCv by all staff and visitors in a human intensive care unit was not helpful in preventing common pathogens or in decreasing length of stay, nor did ShCv have any significant effect on mortality.¹

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A recent technology, contamination control flooring (CCF), has been marketed as an alternative strategy to ShCv. In research animal facilities, CCF is becoming a popular alternative to disposable ShCv due to its proposed longevity of 3 to 5 y, ability to be recycled after it expires, reduction in landfill waste, cost-effectiveness, and increased energy efficiency.²³ One study comparing the effectiveness of adhesive mats, CCF, and ShCv found that either ShCv or CCF was better than adhesive mats for decreasing the organic load on animal room floors; however, none of the methods evaluated resulted in significantly decreased bacterial contamination of floors.²

For the current study, we sought to determine the effect of various floor contamination control methods on not only the prevention of bacterial agent transfer and reduction of organic load on the floor but also on the presence of murine pathogens in the environment and effect on colony animal health status. We examined the effects of 4 floor contamination control methods—ShCv, CCF, ShCv+CCF, and as well as neither product—on the prevention of bacterial transfer into facilities, the reduction of organic load between the facility entrance and animal housing area, and the presence of excluded and nonexcluded murine pathogens in rodent IVC facilities. We hypothesized that using ShCv+CCF would prevent the transfer of bacteria most effectively and reduce the organic load on facility floors. We also considered that floor contamination control method would have no effect on the presence of pathogens on floors nor would result in any change in colony excluded pathogen status as measured by surveillance testing.

Materials and Methods

Animal facilities. Studies were conducted in animal facilities containing at least one room housing mice in IVC at the University of North Carolina at Chapel Hill (Figures 1 and 2). The average rodent IVC census for each facility ranged from 300 to 12,500 cages, and approximate facility size (including both animal housing and support space) ranged from 2800 to 30,000 ft². The distance between the facility entrance and animal housing room in all facilities ranged from 6 to 124 ft. PPE in any single facility (other than experimental floor contamination control conditions in the second cohort of the organic load testing experiment) remained consistent throughout the study. Each rodent housing room used at least one mobile animal transfer station, with room-supplied HEPA-filtered air, in which cages were changed and animals handled. On the same 2 afternoons each week, husbandry staff mopped epoxy-finished facility floors by using a quaternary ammonium chloride disinfecting liquid (Vimoba 128, Quip Laboratories, Wilmington, DE). Shoe covers (ShCv; Medline, Northfield, IL) made of water-resistant medium-weight coated polypropylene were supplied inside the entrance doors of each animal facility. During the study, facility users were instructed to don ShCv on entering the facility and to remove them on exit. Facility entry was granted through keycard access, and electronic permissions were provided to husbandry, veterinary, and research staff.

Contamination control flooring (CCF; TechTrak, Coventry, RI) was installed at PPE-donning stations and along facility corridors in areas of high traffic, such as outside the entrance to cage wash. CCF mats are professionally installed semipermanently and are designed to last 3 to 5 y. These high-tack polymer mats have a liquid smooth surface for increased contact with shoe or wheel surfaces. Due to the conforming property of the polymer and its concentrated loading of particles, the manufacturer claims that mats are effective after continuous overstrikes in the same traffic area.²³ The mats used in this study contain a

silver-based antimicrobial that inhibits the growth of bacteria on shoe surfaces²³ and that attracts and collects particles from shoe soles, wheels, and air.^{6,19,21,23} A minimum of 3 foot strikes or wheel turns are required for correct use, and the manufacturer recommends daily cleaning with a disinfectant, followed by removal of the cleaning solution by squeegeeing. Husbandry staff received instruction regarding the correct use and disinfection of mats. To ensure compliance among all facility users, all mats were custom-sized and installed to ensure that the minimum (at least) effective foot and wheel contact was achieved. In each facility, mats extended across the full width of the corridor and ranged from 10 to 61 ft in length.

The effect of floor contamination control method on organic load and murine pathogen presence was evaluated in 2 facility cohorts, each consisting of 4 facilities. In the first cohort (labeled facilities 1 through 4; Figure 1), each animal facility used 1 of 4 floor contamination control methods throughout the 6-wk study: facility 1, ShCv only; facility 2, CCF mats; facility 3, ShCv+CCF mats; and facility 4 neither ShCv nor CCF mats. In the second cohort of animal facilities (labeled facilities A through D, Figure 2), the floor contamination control method was changed every 4 wk throughout the course of the study; each facility used ShCv only for 4 wk, then ShCv+CCF mats for 4 wk, followed by CCF mats only for 4 wk. One facility was used in both cohorts (facility 1 [Figure 1] and facility D [Figure 2]).

Sentinel animals. CD1 mice purchased from Charles River Labs (Wilmington, MA) or Swiss Webster mice propagated through an inhouse breeding program were used as sentinels. Mice were free from epizootic diarrhea of infant mice virus, Theiler murine encephalomyelitis virus and the GDVII strain of that virus, mouse hepatitis virus, mouse parvovirus, minute virus of mice, mouse norovirus, mouse cytomegalovirus, mouse adenovirus 1 and 2, polyoma virus, pneumonia virus of mice, reovirus 3, ectromelia virus, lymphocytic choriomeningitis virus, Sendai virus, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus, fur mites (*Myobia musculi*, *Radfordia affinis*, and *Mycoptes musculus*), and pinworms (*Aspicularis tetraptera*, *Syphacia muris*, *Syphacia obvelata*). Two mice were housed in each IVC (Tecniplast, Milan, Italy) according to recommendations in the *Guide for the Care and Use of Laboratory Animals*¹⁰ on a 12:12-h light:dark photoperiod, temperature of 70 to 72 °F (21.1 to 22.2 °C), and relative humidity of 30% to 70%. Mice were observed daily by husbandry staff and had unrestricted access to irradiated or autoclaved feed (Teklad 2920X or 2020SX, Envigo, Indianapolis, IN) and reverse-osmosis-purified, chlorinated water delivered by an automatic watering system. One heaping teaspoon of dirty bedding with feces from each cage on one side of a rack (maximum, 79 cages) was added to the sentinel cage, with cage changes occurring biweekly. Blood was collected from the submandibular vein for serologic testing by using OPTI-Spot strips (IDEXX BioResearch, Columbia, MO) every 4 mo as part of the standard facility mouse surveillance program, with one sample collected before installation of CCF and a second sample collected at least 2 mo after CCF installation. Serologic testing included evaluation for antibodies against epizootic diarrhea of infant mice virus, mouse hepatitis virus, mouse parvovirus, minute virus of mice, and Theiler murine encephalitis virus. Only samples from sentinels exposed to dirty bedding from investigators' animals that were housed in the facilities throughout the entirety of the study were included in this experiment. The number of sentinels tested for facility C varied (preCCF, $n = 12$; postCCF, $n = 10$) due to a reduction in the numbers of an investigator's animals during the preCCF sampling period; this reduction led to consolidation of cages on racks during the

	Floor contamination control method	Species housed	Rodent IVC census	Approximate area (ft ²)	PPE worn
Facility 1	ShCv only	mice, rats	300	3600	coveralls, gloves, bonnet, surgical mask
Facility 2	CCF only	mice, rats	1700	11,000	gown, gloves
Facility 3	ShCv and CCF	mice	12,500	30,000	coveralls, gloves, bonnet, surgical mask
Facility 4	none	mice, rats, guinea pigs, rabbits, ferrets, swine, macaques	800	20,000	gown, gloves

Figure 1. Description of the first cohort of rodent IVC facilities tested. Approximate square footage includes both animal housing space and support or procedural space.

	Species housed	Rodent IVC census	Approximate area (ft ²)	No. of rooms sampled	PPE worn
Facility A	mice, rats, gerbils	1000	6000	2	coveralls, gloves, bonnet, surgical mask
Facility B	mice, rats	1700	7300	2	coveralls, gloves, bonnet, surgical mask
Facility C	mice, rats	500	2800	2	gown, gloves
Facility D	mice, rats	300	3600	1	coveralls, gloves, bonnet, surgical mask

Figure 2. Description of the second cohort of rodent IVC facilities tested. Approximate square footage includes both animal housing space and support or procedural space.

study. Serologic samples collected as part of the preCCF cohort represent the health status of the animals housed in each facility prior to any experimental manipulation of floor contamination control. All studies involving animals were approved by the IACUC of the University of North Carolina at Chapel Hill, an AAALAC-accredited institution.

Bacterial transfer experiment. *Staphylococcus xylosus*, an environmentally persistent commensal bacterial strain^{15,20} found on the skin of humans and animals, was collected from the skin of 2 colony mice and cultured on trypticase soy agar with 5% sheep blood (TSA II; Becton Dickinson, Franklin Lakes, NJ). Pure cultures of *S. xylosus* were confirmed by using physical and biochemical methods and then verified through PCR analysis. Bacteria were grown on TSA II plates for 18 to 24 h, after which bacteria were emulsified in sterile distilled water to be equivalent to 1.2×10^9 cfu/mL, or McFarland Standard Number 4.

We then pipetted 2 mL (1.2×10^9 cfu/mL) *S. xylosus* suspended in sterile distilled water onto the soles of closed-toe shoes of various styles, sizes, and tread; we evenly distributed the suspension over the sole by using a cotton-tipped applicator and then allowed the shoes to air dry. Volunteers donned the shoes, stood in a demarcated area, and walked to a second demarcated area 16 ft away while using 1 of the 4 contamination control methods: ShCv; CCF mats; (3) ShCv+CCF mats; and (4) neither product (that is, bare shoes on the facility floor). Three strikes with each foot were made between the first and second demarcated areas. Although generally considered nonpathogenic and not excluded from institutional animal facilities, *S. xylosus* has been reported to cause opportunistic infections in immunocompromised mice;^{5,8,21} therefore, the experiment was conducted in an animal facility that already had CCF mats installed but did not house rodents, and all surfaces were thoroughly disinfected at the conclusion of the experiment.

Each pair of shoes was used with each floor contamination control method during the same experimental period, with one shoe undergoing PCR testing and the other for culturing. Prior to the start of the experiment, between trials, and after completion of the experiment, shoe soles and floor sampling areas were disinfected with Vimoba 128 (Quip Laboratories) for a minimal contact time of 10 min, followed by 70% isopropyl alcohol for a minimal contact time of 5 min. Before the start of the experiment, between trials, and after completion of the experiment, CCF mats and corridors were disinfected by using Vimoba 128 (Quip Laboratories) and a sponge mop and then squeegeed (CCF mat) or allowed to air dry (corridor floors).

Swabs were collected from the demarcated areas on the floor located at the start and at the end of the floor contamination control condition. Negative controls were collected from disinfected floor sampling areas at the conclusion of each experiment, to confirm that the disinfection strategy was effective at eliminating *S. xylosus* on floors; positive controls were obtained directly from the bacterial culture media. Swabs collected from the left-foot floor areas were submitted to IDEXX BioResearch to quantitate the number of *S. xylosus* genome copies. Swabs consistently collected from demarcated areas on the floor for the right foot were streaked onto a TSA II plate. *S. xylosus* colonies were counted manually after 2 and 3 d of incubation at room temperature. Data are presented as the *S. xylosus* copy number (or cfu) from the finish floor-sampling area divided by the *S. xylosus* copy number (or cfu) from the start floor-sampling area. For one set of shoes, a negative PCR result was obtained at the start sampling area for the 4th contamination control method (neither ShCv nor CCF) despite a high *S. xylosus* copy number from the finish sampling area and high *S. xylosus* colony count identified through culture from both the start and finish floor-sampling areas for the same shoes. Because of this discrepancy, the PCR data obtained from this set of shoes for all 4 contamination control methods were excluded from the analysis ($n = 7$), although the culture data collected from these shoes were retained in analyses ($n = 8$). Data are pooled from 2 independent experiments.

Organic load testing. Measurements for ATP were collected by using a rapid ATP swab (PocketSwab Plus, Charm Sciences, Lawrence, MA) and immediately analyzed by using a handheld luminometer (novaLUM II, Charm Sciences). The amount of ATP present in each of the samples was measured in relative light units (RLU). To provide consistency in the amount of floor space sampled from each facility, a polycarbonate square (232.26 cm²) was placed on the floor for sampling and used as a guide. The plastic square was disinfected with Vimoba 128 (Quip Laboratories) between facilities. Floor samples were collected the day before floors were cleaned to ensure that an accurate sampling of the organic load present on the floor was obtained.

In the first cohort of facilities (facilities 1 through 4), samples were collected at 3- or 4-d intervals over 6 wk ($n = 11$) at 4 locations within each facility: 1) the sample representing the facility entrance was taken at the badge access point just prior to entering the animal facility; 2) the postPPE sample from the donning area was collected from the corridor floor immediately off the contamination control mat or immediately after the PPE

donning area opposite of the facility entrance; 3) the sample representing the animal room entrance was collected from the floor just before the entrance to the animal room; and 4) the animal room change station sample was collected inside the animal housing room on the floor immediately in front of the mouse cage-changing station. All animal rooms tested in this cohort housed mice in hot-water-washed, nonautoclaved cages.

In the second cohort of facilities (facilities A through D), floor samples were collected from the same facility under different subsequent floor contamination control conditions: ShCv only, ShCv+CCF mats, CCF mats with twice-weekly mopping, and CCF mats with daily cleaning by using a squeegee. Samples were collected from all 7 rooms across all 4 facilities at 3- or 4-d intervals so that sampling coincided with the days prior to scheduled biweekly mopping of facility floors ($n = 8$ repeated samples) from 3 locations within each facility. In particular, the sample from the facility entrance was collected at the badge access point just prior to entering the animal facility; the sample for the animal room entrance was obtained from the floor next to the door to the room; and swab sample for the animal room change station was made inside the animal housing room on the floor immediately in front of the mouse cage changing station. Sampling locations for the second cohort of facilities excluded the postPPE donning area described in the first facility cohort to avoid areas of floor to be covered by CCF mats. For facilities A, B, and C, samples were collected from 2 animal rooms; in facility D, only one mouse housing room was accessible for evaluation of ATP levels throughout the study; therefore, room entrance and change hood samples were collected from the single room only. When CCF mats only were in use, they were mopped biweekly for 4 wk and then squeegeed daily for 4 wk; samples were collected from the animal room entrances and change stations in 2 rooms each in facilities A, B, and C ($n = 6$) over 8 repeated samples during each cleaning method period. Data collected during the 4 wk of biweekly mopping were included in both floor contamination control method and CCF mat cleaning analyses.

Assessing floors for murine pathogens by using PCR analysis. PCR analysis was used to evaluate the presence or absence of common environmentally persistent murine pathogens in facilities using various floor contamination control methods. A flocked swab (PurFlock Ultra, Puritan Medical Products, Guilford, ME) was used to collect a sample from the floor at 2 locations in each facility—the badge access point just before the entrance to the animal facility (facility entrance swab) and immediately in front of the cage-changing station (animal room swab)—from the first cohort of facilities (facilities 1 through 4). To provide consistency in the amount of floor space sampled from each facility, a polycarbonate square (approximately 230 cm²) was placed on the floor and used as a guide for sampling; the plastic square was disinfected by using Vimoba 128 (Quip Laboratories) between facilities. For 6 wk, floor samples were collected once each week on the day before floors were cleaned, to ensure that the pathogens present on the floor were sampled accurately. Swabs were submitted to IDEXX BioResearch for PCR testing for murine pathogens that are excluded or nonexcluded as part of the standard mouse health surveillance program at our institution. Excluded pathogens included pinworms (*Aspiculruis tetraoptera*, *Syphacia muris*, *Syphacia obvelata*), mouse hepatitis virus, and mouse parvovirus, and nonexcluded pathogens included mouse norovirus, *Corynebacterium bovis*, and *Helicobacter* spp. (*H. bilis*, *H. ganmani*, *H. hepaticus*, *H. mastomyrinus*, *H. rodentium*, and *H. typhlonius*).

Exhaust air dust sampling. To evaluate the effect of floor contamination control method on mouse IVC colony health status, exhaust air dust (EAD) was sampled from racks in the second cohort of facilities (facilities A through D). Prior to the study, animal housing racks were disassembled and cleaned by completing a hot wash cycle (temperature reaching 180 °F [82.2 °C]) for at least 3 min. Racks for rooms with sterile caging were autoclaved after the wash cycle. Samples were collected from the exhaust air filter by using a flocked swab and pooled from all racks within in each facility; samples were obtained every 4 wk coinciding with each change in contamination control method (ShCv, followed by ShCv+CCF, and then CCF). EAD samples were collected after each contamination control method had been in place for 4 wk to ensure ample time for the deposition of EAD in filter pans. Because ShCv were already in use in facilities A through D at the start of the study and because no other changes in PPE were made, results from the ShCv samples represent the health status of animals prior to any changes in floor contamination control method. EAD samples were submitted to IDEXX BioResearch for PCR testing for murine pathogens that are excluded as part of the standard institutional mouse health surveillance program. Examined pathogens included pinworms (*Aspiculruis tetraoptera*, *Syphacia muris*, *Syphacia obvelata*), fur mites (*Mycoptes* spp., *Myobia* spp., and *Radfordia* spp.), and mouse hepatitis virus.

Statistical analysis. All graphs were prepared and statistical analyses performed by using Prism 7.0 (GraphPad Software, La Jolla, CA). *Staphylococcus xylosus* PCR and colony count ratios were examined by using a nonparametric Friedman test with Dunn multiple comparisons posttest to determine statistical differences among floor contamination control methods. ATP levels in the first cohort of buildings (facilities 1 through 4) were analyzed by using 2-way repeated-measures ANOVA with a Dunnett posttest to compare levels within the facility with those at the facility entrance. Longitudinal ATP level ratios collected from animal room entrances or change stations in the second cohort of facilities were analyzed by using 2-way repeated-measures ANOVA. A Tukey multiple-comparisons posttest was used to compare the ATP ratios over the repeated sampling and to determine statistically significant differences between floor contamination control methods and floor cleaning methods. A *P* value of less than 0.05 was considered significant in all analyses.

Results

Effectiveness of various floor contamination control methods in preventing bacterial transfer onto facility floors by means of a user's shoes. An animal facility user's shoes represent a possible source of pathogen introduction onto facility floors. To evaluate the effectiveness of various floor contamination control methods in preventing bacteria from a user's shoes from being deposited onto the floor upon entering a facility, we applied *S. xylosus* to the bottoms of shoes and quantified bacterial numbers by PCR and culture analyses prior to and following the use of 1 of 4 floor contamination control methods. We selected *S. xylosus* because it is an environmentally persistent commensal organism that is readily culturable.

S. xylosus readily transferred from shoe soles to facility floors, and bacterial DNA on the floors at the start sample location was readily detected through PCR analysis (Figure 3A). The relative reduction in *S. xylosus* copy number was found to be significantly different ($P = 0.0003$) between various floor contamination control methods tested. *S. xylosus* PCR results were negative for all floor samples collected at the finish location when ShCv were used, whether alone or in conjunction with CCF mats. Relative

Figure 3. Effectiveness of various floor contamination control methods in preventing the transfer of *Staphylococcus xylosus* from users' shoes to the floor. *S. xylosus* was applied to the soles of shoes before crossing a stretch of facility flooring while using one of 4 floor contamination control methods: shoe covers only (ShCv Only), contamination control flooring mats only (CCF), both shoe covers and contamination control mats (ShCv+CCF), and no floor contamination control method (none). (A) Relative *S. xylosus* copy numbers according to PCR analysis and (B) colony counts from cultures from floor sampling areas immediately before and immediately after the use of each floor contamination control method were evaluated. Data are presented as the *S. xylosus* copy number or colony count (in cfu) at the sampling location immediately after applying the floor contamination control method (finish location) divided by the *S. xylosus* copy number or colony count (in cfu) at the sampling location immediately before applying the floor contamination control method (start location) and are presented as the median $\pm$ interquartile range; $n = 7$ or 8 floor samples per floor contamination control method. *, $P < 0.05$, Friedman test with Dunn post hoc test for multiple comparisons test for intergroup comparisons.

S. xylosus copy numbers for CCF mats alone (median, 1.273) and neither ShCv nor CCF mats (median, 1.017) were both greater than 1, indicating these methods were ineffective in reducing bacterial numbers below detectable limits. Relative *S. xylosus* copy numbers were significantly ($P = 0.003$) higher when CCF mats or neither ShCv nor CCF mats were used compared with when ShCv or ShCv+CCF were used.

In addition to evaluating the quantity of *S. xylosus* by PCR, we cultured floor samples and analyzed the relative colony count of *S. xylosus* (Figure 3 B). *S. xylosus* was readily cultured from all samples collected from the floor at the start location, and the relative colony count of *S. xylosus* differed significantly ($P = 0.0012$) among the floor contamination control methods. No *S. xylosus* was cultured from the floor at the finish location when ShCv only were used; when ShCv+CCF was used, 6 of the 8 samples collected at the finish location were negative by culture for *S. xylosus*, and the 2 positive samples yielded only a single *S. xylosus* colony each. In contrast, *S. xylosus* was cultured from 6 of the 8 floor samples collected at the finish location after the use of CCF only (median relative number of *S. xylosus* colonies, 0.625 cfu) and from all samples collected at the finish location when neither ShCv nor CCF mats were used (median relative number of *S. xylosus* colonies, 0.383 cfu). A significantly higher relative colony count of *S. xylosus* was found when CCF mats alone or when neither ShCv nor CCF mats were used compared with when ShCv were used alone. These findings show that floor bacterial transfer was most effectively prevented when ShCv were employed.

Effect of floor contamination control method on the reduction of organic load on the floors of rodent IVC facilities. Organic load

is commonly assessed by ATP bioluminescence meters in laboratory animal facilities as a quick and convenient measurement for cleaning and sanitation efficacy.²⁴ By using a luminometer, ATP levels on the floor were quantified in 4 facilities using different contamination control methods (facilities 1 through 4) at the facility entrance and 3 areas within the animal facility (Figure 4). A significant difference in ATP levels was seen among sampling locations within the facility ($F_{3,120} = 29.3$, $P < 0.0001$) but not floor contamination control methods ($F_{3,40} = 1.006$, $P = 0.4003$). A significant reduction in ATP level was seen between the facility entrance and each of the intrafacility sampling locations in the facilities using ShCv, CCF, and ShCv+CCF methods, with the most dramatic reduction seen in the facility using ShCv+CCF. Although not statistically significant, an approximately 2-fold reduction in ATP levels was seen between the facility entrance and the intrafacility locations in the facility that used neither ShCv nor CCF (facility 4). This finding indicates that factors other than the floor contamination control method, such as inherent differences between facilities or traffic patterns within facilities, affected ATP levels.

To examine the effectiveness different floor contamination control methods had on reducing ATP levels within the same facility, organic load on facility floors was next evaluated in mouse IVC housing rooms located in 4 facilities ($n = 7$ rooms, facilities A through D) over 8 repeated samples (Figure 5). The floor contamination control method was changed every 4 wk, starting with ShCv, followed by ShCv+CCF, and then CCF. At both the animal room entrance (Figure 5 A) and change station (Figure 5 B), repeated-measures 2-way ANOVA revealed a statistically significant effect of floor contamination control

Figure 4. Organic load on floors in various locations of rodent IVC facilities using various contamination control methods. ATP levels on the floor of 4 facilities, each using a different contamination control method, were measured at the facility entrance (black solid circle), after the PPE donning area (white open square), at the animal room entrance (gray solid triangle), and at the animal room change station (gray open diamond). Black horizontal line represents the data mean; $n = 11$ samples per floor contamination control method per location within each facility. *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$; §, $P < 0.0001$ (2-way repeated measures ANOVA with Dunnett posthoc test for multiple comparisons) and ns, not significant.

method on ATP levels ($F_{2,18} = 14.82$, $P = 0.0002$ for animal room entrance; $F_{2,18} = 10.18$, $P = 0.0011$ for animal room change station). In addition, most variation in ATP levels occurred at the room level, because individual rooms had significant matching, thus demonstrating consistent ATP levels within each room for each floor contamination control method over the 8 repeated samples collected ($F_{18,126} = 2.830$, $P = 0.0004$ for animal room entrance; $F_{18,126} = 4.188$, $P < 0.0001$ for animal room change station). At both the animal room entrance and the change station, the greatest reduction in ATP levels was seen when ShCv+CCF were used (animal room entrance mean: ShCv, 0.25 RLU; ShCv+CCF, 0.13 RLU, and CCF only, 0.44 RLU; animal room change station mean: ShCv, 0.18; ShCv+CCF, 0.11 RLU; and CCF, 0.53 RLU). Statistically significant ($P = 0.03$) reductions in organic load were seen for the CCF only condition compared with the ShCv and ShCv+CCF conditions at both the animal room entrance and change stations. In summary, CCF mats were the floor contamination control method that was least effective at reducing the organic load on facility floors.

The manufacturer of the CCF mats recommends daily cleaning and squeegeeing of the mats to maximize particle removal from shoes and equipment, but this process involves additional personnel effort beyond the time needed to mop the floors of a facility. To determine whether floor disinfection routines affected the reduction in organic load on facility floors, CCF mats in mouse IVC housing rooms located in 3 facilities ($n = 6$ rooms; facilities A, B, and C) were either mopped biweekly along with the rest of the facility floors or were cleaned and squeegeed daily according to manufacturer instructions. ATP levels were measured in 8 repeated samples at the animal room entrance (Figure 6 A) and animal room change station (Figure 6 B). Cleaning method did not affect the reduction in ATP levels at either sampling location (animal room entrance: $F_{1,10} = 2.619$, $P = 0.1366$; animal room change station: $F_{1,10} = 0.3285$, $P = 0.5792$). These results suggested that daily squeegeeing was not superior to biweekly mopping for CCF mats for cleaning facility floors to reduce organic load.

Floor surveillance testing of excluded and nonexcluded murine agents in rodent IVC facilities using different floor contamination control methods. ATP measurements are useful for understanding the effectiveness of different floor contamination control methods

in enhancing overall surface cleanliness but are not specific for the various infectious agents that can affect research outcomes.^{18,24} After determining that ShCv, whether in combination with CCF flooring or alone, were most effective in preventing the transfer of an infectious agent from a user's shoes to animal facility floors, we next asked whether murine pathogens were present on the floors of active rodent IVC facilities at our institution, thereby serving as a potential source of adventitious infections to resident mice. Samples ($n = 6$) were collected from the floors at the facility entrance and within an animal housing room over the course of 6 wk in 4 buildings, each using a different floor contamination control method (facilities 1 through 4) to survey the presence of several excluded and nonexcluded murine pathogens. A diverse group of pathogens with different levels of environmental persistence and susceptibilities to commonly used surface disinfectants were selected for testing and included helminths (*Aspiculuris tetraptera*, *Syphacia muris*, *Syphacia obvelata*), an enveloped virus (mouse hepatitis virus), nonenveloped viruses (mouse parvovirus and mouse norovirus), and bacteria (*Corynebacterium bovis* and *Helicobacter* spp.). No excluded pathogens (murine pinworms, mouse hepatitis virus, mouse parvovirus) were detected during the duration of the experiment in any location. Nonexcluded pathogens (mouse norovirus and *Helicobacter* spp.) were sporadically detected in facilities; these included mouse norovirus detected in the animal room in the facility using ShCv+CCF and mouse norovirus and *Helicobacter typhlonius* in the animal room in the facility using neither ShCv nor CCF mats. In summary, surveillance of 4 active rodent IVC facilities over a 6-wk period at our institution did not reveal any of the evaluated pathogens on the floors outside of the facilities, and the presence of nonexcluded pathogens on the floors within animal rooms was not affected by floor contamination control method.

Effect of floor contamination control method on resident mouse health status. Floor contamination control method did not affect the presence of several infectious organisms on the floors within animal rooms, and we next evaluated whether changing the method affected the health status of mice housed in IVC facilities. The presence of excluded parasitic and viral murine pathogens (pinworms, fur mites, mouse hepatitis virus) in EAD samples collected from 4 rodent IVC facilities (facilities A through D) was evaluated by PCR analysis at the end of each 4-wk period of use of ShCv, ShCv+CCF, or CCF. EAD sampling detected no excluded pathogens at any time in any of the facilities, regardless of the floor contamination control method in use.

Although EAD testing has become a standard method for evaluating the presence of many pathogens of mice housed in IVC facilities,^{4,12,26} the use of dirty-bedding sentinels remains the 'gold standard' for monitoring for the presence of select murine pathogens, including mouse parvovirus.¹¹ To further evaluate the effect of floor contamination control method on colony health status, serologic samples from sentinel mice housed in 4 rodent IVC facilities (facilities A through D) were collected prior to CCF mat installation and at 8 to 16 wk afterward; 5 excluded viral murine pathogens (epizootic diarrhea of infant mice virus, mouse hepatitis virus, mouse parvovirus, minute virus of mice, and Theiler murine encephalomyelitis virus) were evaluated through PCR analysis of each serologic sample. Testing 35 samples collected before CCF installation and 33 samples afterward across the 4 facilities yielded findings similar to those from EAD swabs, such that none of the viral pathogens were detected at any point during the study in any of the facilities. Together with the results from EAD sampling, dirty-bedding sentinel testing showed—in a real-world setting—that changing the floor contamination control method did not result in the

Figure 5. Effect of floor contamination control method on reduction of floor ATP levels within rodent IVC facilities. The ATP level (in RLU) of the (A) animal room entrance or (B) animal room change station relative to that of the facility entrance was measured for 3 floor contamination control methods used over 8 repeated samples. Data are presented as mean $\pm$ SEM; $n = 7$ rooms per floor contamination control method (ShCv Only, shoe covers only; ShCv+CCF, both shoe covers and contamination control flooring; CCF Only, contamination control flooring mats only). *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ (2-way repeated measures ANOVA with Tukey posthoc testing for multiple comparisons for intergroup comparisons; the dashed black vertical line represents ShCv+CCF compared with CCF Only).

introduction of selected excluded pathogens to mice housed in IVC facilities.

Discussion

Healthy animals of known pathogen status are a necessity for the reproducibility and reliability of research results, and biosecurity represents one way to achieve optimal rodent health status, through the exclusion of specific pathogens from animal facilities. Because human activity may be responsible for accidental pathogen introduction into animal facilities, it is necessary to evaluate how to best mitigate these risks. In our facilities, animal–human contact is limited due to housing animals in IVC, restricting the opening of cages to inside of a laminar-flow hood, and handling animals while wearing gloves sprayed with an oxidizing disinfectant (Virkon, Cologne, Germany). In the event of transmission of a murine pathogen from a shoe onto the floor, the most likely way that the pathogen could infect animals is through nonadherence to standard operating procedures for housing and handling mice. Floor contamination control is one biosecurity measure that can be used to potentially control and contain murine pathogens. In this study, we evaluated 4 contamination control methods regarding their ability to prevent the introduction of a bacterial agent into a facility via users' shoes and to reduce organic load on facility floors as well as their effect on the health status of colony mice. We found that CCF was less effective than donning new ShCv for preventing bacterial transfer and reducing organic load on the floors of animal facilities. However, when we evaluated the

presence of excluded murine pathogens through surveillance testing, floor contamination control method did not result in any changes to animal health status.

We examined how floor contamination control method affects the relative reduction in organic load from facility entrance to animal housing rooms in rodent IVC facilities. Interestingly, there was an approximately 50% (albeit nonsignificant) reduction in ATP levels from facility entrance to all areas tested within the facility when neither ShCv nor CCF was used (facility 4), suggesting that other factors affected the amount of organic load on the floors. One explanation for this reduction may be the pattern of foot traffic; because facility entrance floor sampling areas were located at badge access areas, foot traffic is heaviest at this sampling area, consistent with the higher levels of ATP measured. Because users travel to multiple end locations within a facility, the farther within the facility an animal room is located, the less foot traffic it experiences, and therefore the less organic load transfer will occur on the floors. As facility visitors walk through an animal facility where no floor contamination control method is in use, organic material from their shoes may become dispersed within the facility, resulting in a reduction in ATP levels. In addition, the relative magnitude of organic load detected in animal facilities appears to be affected by season and other environmental factors, given that we measured higher ATP levels on the floors in facilities that corresponded to peak pollen season in the geographic area where the experiment was performed. In addition, variations in floor cleaning practice

Figure 6. Effect of contamination control floor mat cleaning method on reduction of floor ATP levels within rodent IVC facilities. The ATP level (in RLU) of the (A) animal room entrance or (B) animal room change station relative to that at the facility entrance was measured over 8 repeated samples while CCF mats were either mopped biweekly or squeegeed daily. Data are presented as mean $\pm$ SEM; $n = 6$ rooms per floor contamination control method.

could account for the amount of organic debris on floors. Regardless, no overt differences in organic load were observed on floors during any of the floor contamination control conditions.

Although reduction of organic load is considered the standard for overall cleanliness of a facility, pathogens present on facility floors—whether tracked in from outside the facility by a user's shoes or through contamination by materials or equipment that have come in contact with positive animals currently housed in a facility—represent a possible source of infection to naïve colony animals when other contamination control methods are not in practice. Using a real-world approach, we surveyed the presence of several excluded and nonexcluded pathogens both outside and within animal rooms of 4 active rodent IVC facilities at our institution and sought to determine whether the floor contamination control method used affected this presence within animal rooms. Throughout the 6-wk study, no murine pathogens excluded from our institution were ever detected on the floors inside or outside of any facility. However, positive PCR results for mouse norovirus, a nonexcluded pathogen, were detected for the floors inside animal rooms but not the floors located outside the facilities of the facility using ShCv+CCF and that using neither ShCv nor CCF, even though these facilities demonstrated the most disparate reductions in ATP levels from the facility entrances; this finding suggests that contamination of the floors with mouse norovirus was due to materials coming into contact with pathogen-positive animals (for example, spilled soiled bedding) instead of floor contamination being introduced from outside the facility due to facility users' shoes. Although the viability of these pathogens was not evaluated in this study, environmental contamination with nonexcluded agents already present in a subset of colony animals represents a possible source of infection for naïve animals located in the same room. Therefore, husbandry staff and laboratory personnel should be trained to not place caging or equipment that may come in contact with animals on the floor, to change their gloves and other contaminated PPE before handling animals when they touch the floor or their shoes, and to appropriately disinfect equipment and change station surfaces between cages of mice.

To our knowledge, the effect of floor contamination control method on rodent health status has not been evaluated previously, and for this study, we sought to determine whether

changing the floor contamination control method resulted in the introduction of excluded pathogens to colony animals. To gain the most information about how different contamination control methods affect the excluded pathogen status of mice housed in IVC facilities at a large academic institution, we conducted our experiments in occupied animal rooms located in multiple facilities. Although conducting experiments under more controlled conditions could have reduced the influence of confounding variables, our real-world study design allowed for facility-typical foot traffic that would not have been possible in controlled conditions, where animals would be housed on a separate animal housing rack or possibly in a separate room. Foot traffic was an important consideration when designing this experiment because we were curious whether facility users were tracking murine pathogens from outside of the facility to the inside of animal facilities and especially inside of animal housing rooms. In addition, our experimental design allowed us to conduct these experiments without using any additional animals, a feature that supports the 3Rs. However, because of limitations inherent to our real-world study design, our conclusions regarding the effect of floor contamination control method on animal health status are restricted to observations involving a specific set of excluded pathogens and cannot be extended to other pathogens or infectious agents that may be already present in facilities. To definitively determine whether floor contamination control method affects the disease status of colony animals, a controlled study that involves keeping animals of a defined health status in a clean, isolated location and introducing specific agents to the facility floors by means of a user's shoes followed by EAD or sentinel testing for that specific agent would need to be performed. In addition, we tested only a limited number of murine pathogens in this study, and to fully evaluate the efficacy of different floor contamination control methods, it would be beneficial to examine additional murine pathogens, use methods such as 16S PCR to detect the microbial diversity or further characterize the bacterial burden on facility floors,^{13,14,17,22} or to search for pathogens natural to other species, such as rats. Effects beyond organic load and colony health status as evaluated by surveillance testing that were not examined in this study, such as the microbiome, could also be affected by floor contamination control method

and represent an area of future study. Regardless, our results suggest that with all the engineering controls in place in rodent IVC facilities, such as the use of laminar flow hoods, other PPE choices and practices, through health surveillance procedures, and strict quarantine measures of imported animals, the floor contamination control method has minimal effect on the health status of animals housed in research facilities.

Despite the increasing popularity of CCF mats in research animal facilities, there is a large upfront cost associated with their purchase and installation; in addition, the manufacturer recommends at least once-daily sponge mopping and squeegeeing with a disinfectant, requiring considerable personnel time for general cleaning and upkeep of CCF mats. Furthermore, we found that these mats become very slippery during mopping and squeegeeing, potentially increasing the risk of slips and falls in the workplace, especially as the daily frequency of squeegeeing increases. In contrast to the large upfront cost of CCF, ShCv costs are continuous and substantial but largely dependent on foot traffic volume. Our findings suggest that floor contamination control methods do not markedly affect the health status of mice housed in IVC facilities and are not necessary for general mouse IVC housing at our institution. However, if floor contamination controls are necessary or desired for biocontainment, such as in ABSL2 or 3 facilities, or in facilities requiring an exceptionally clean environment, such as those maintaining a cleanroom for sterile product production, ShCv used in conjunction with CCF represent the superior contamination control method at decreasing organic load and preventing bacterial transfer on floors of research facilities.

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