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Effectiveness of Shoe Covers for Bioexclusion within an Animal Facility

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The personal protective equipment (PPE) required for entry into rodent barrier rooms often includes a hair bonnet, face mask, disposable gown, gloves, and shoe covers. Traditionally, shoe covers have been considered essential PPE for maintaining a 'clean' animal room. The introduction of microisolation caging and ventilated rack housing prompted us to reevaluate the contribution of shoe covers to bioexclusion. Contamination powder that fluoresces under black light was to track particle dispersal on the floor and personnel. The test mouse room contained a ventilated microisolation rack and biosafety cabinet. Powder was applied directly inside or outside the animal room doorway. PPE with or without shoe covers was donned outside of the animal room doorway and discarded on exiting. Participants either were scanned on entry into the room for the presence of florescence or asked to complete a simulated standard animal room activity while wearing full PPE. Animal rooms were scanned for florescence after exit of participants. All participants donning shoe covers fluoresced in multiple areas, primarily on gloves and gowns. Shoe covers had no effect on the spread of powder in normal traffic patterns, with no powder detected within caging. Powder also was used to determine the distance substances could be carried on the floor from building entry points. Results indicate that shoe covers do not improve (and actually may compromise) bioexclusion. Donning of shoe covers offers a potential for contamination of personnel from contact with shoe bottoms.

Abbreviations: ACLAM, American College of Laboratory Animal Medicine; PPE, personal protective equipment.

The use of shoe covers as personal protective equipment (PPE) has largely been discontinued in human operating rooms as a mechanism of reducing the introduction of bacteria,.[10,13,21] Shoe covers are currently recommended for use in human hospitals only to protect health care workers from blood and body fluid contamination, as required by the Bloodborne Pathogens Standard.[13,15,16] Little information is available on recommendations for shoe covers in veterinary hospitals. The Canadian Committee on Antibiotic Resistance recommends the use of disposable shoe covers in veterinary hospitals when dealing with "some" patients with infectious diseases to prevent the spread of infectious material present on the floor.[3] However, data to support the efficacy of shoe covers to prevent infection in equine isolation wards are inconclusive.[18]

The use of shoe covers in the laboratory animal setting remains an active topic of discussion. At the 2010 American College of Laboratory Animal (ACLAM) forum, a questionnaire on the types of PPE required at different animal facilities revealed that 83% of 178 respondents required foot protection such as shoe covers or dedicated shoes when manipulating rodents. The majority of survey respondents (76%) felt that shoe covers protected the animals from microorganisms on the wearer, and a smaller subset (38%) thought that shoe covers offer some protection to the wearer. However, despite the overwhelming use of shoe covers as a standard within rodent barrier facilities, some survey respondents noted that shoe covers may represent a source for contamination of hands and that tracking of infectious agents from the floor to the inside of the cage was unlikely.[2] The purpose of the ACLAM PPE questionnaire was to generate discussion regarding current best practices for the use of PPE within laboratory animal facilities. Although this survey demonstrated a clear bias toward the use of shoe covers, this bias appears to be based largely on historical practices. There are no data in the literature to support the use of shoe covers as a method to protect against disease outbreaks.

University Laboratory Animal Resources at The Ohio State University currently requires husbandry staff to change into scrubs before working in the vivaria, and the minimum PPE requirement for a sterile or barrier room had been a disposable gown, shoe covers, face mask, hair bonnet, and gloves. Specific room PPE requirements are determined based on the health status of the animals in the room and are posted on the outside door, and required PPE must be donned by all personnel before entering. Personnel may be required to shower before entry into high-risk areas such as sterile rodent rooms.

A recent review of the current institutional policy on PPE revealed that there were few data to support the need for shoe covers for the protection of animals or personnel when working with microisolation cages, ventilated racks, and biosafety cabinets. The current studies were designed to determine the ability of particles on the floor to have an effect at the level of the cage, the ability for particles to access the animal room from outside the room, and the potential for shoe covers to act as a source of contamination for hands and gowns. Contamination powder, which is easy to use, has virtual opacity under visible lighting, is of an appropriate size, and is inexpensive, was used to simulate contaminants at floor level. Fluorescent dyes and powders are used frequently in hospital and medical settings to evaluate or teach cleaning and disinfection procedures and to demonstrate environmental contamination.[8,12,19] The powder selected for the current studies is also nontoxic and easy to clean up, leaving no residual material. These studies resulted in the discontinuation of the use of shoe covers for all of our institutional animal facilities, unless shoe covers are specifically indicated according to identified human or animal biohazard risk.

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Materials and Methods

Materials. Krypton Powder and XR7 Contamination Simulation Powder were obtained from Black Light World (Cub Run, KY). Contamination powder is milled at a 325-to-2500 mesh, which approximates a 44-µm final size. In UV or black light, Krypton powder glows bright green, and XR7 powder glows brilliant blue. Standard cloth-type polypropylene shoe covers with nonskid bottoms (Total MRO, Guilford, CT) were used for all studies. All other PPE items were obtained from Total MRO.

Facility. The studies were conducted in 2 separate active animal facilities. The first facility is a rodent-only facility that opened in 2007 and consists of 42 animal rooms and 14,974 ft^2 of housing space. The second animal facility opened in 1959, houses multiple species, and consists of 30 animal rooms and 38,000 ft^2. Both facilities have floors of seamless epoxy aggregate. Restricted access to facilities is controlled in the rodent-only facility at the entrance to the vivarium by a key card system and is restricted at the suite and procedure-room level with stand-alone card-reader locks (for monitoring and restriction of access by personnel). Access in the multispecies facility is controlled at the entrance to the building and again at the vivarium door by a key-card system and at some rooms with stand-alone card-reader locks.

Partcipants. This study was designed to determine best practices for the use of PPE. Participants were volunteers currently employed by the institution. Participants were observed performing regular work tasks as part of their work day. Volunteers were not compensated and were not graded on their performance of tasks. No identifiable information was recorded for any participant.

Animal room tasks. A ventilated mouse rack (model MI140, Super Mouse Rack, Lab Products, Seaford, DE) was used in all experiments. Participants were requested to perform or simulate (in the case of animal-associated tasks) one of the following common tasks, as described: (1) Animal check (observe each animal in the cage); (2) wean mice in cage M5 into cages at I4 and G9; (3) spot change of cages in spaces I4, J3, E7, and F8; (4) stock room (ensure room is stocked with all necessary supplies such as PPE, extra clean cages, cage cards, acetates, cage card holders, water bottles, disinfectant, and disposable bath towels); (5) separate animals in cage H4 into cages at D9 and D10; (6) transfer mice from cages at B7 and C8 to set up breeding pair in cage K2; (7) spot change of cages I3, J3, K4, A10, and E10; (8) flush rack; and (9) move cages from K1 through 3 to N1 through 3 (Figure 1). Cage locations were chosen at random and to represent all areas of the rack. No live animals were used for any study; inanimate objects (for example, ear plugs to represent weanlings) were placed strategically in cages on the rack to coordinate with the animal room tasks that would be simulated.

Study 1: Contamination from the floor to the rack or cage. An animal room not currently in use was equipped with a class II, type A1 biosafety cabinet (Baker, Sanford, MN) and a ventilated mouse rack (described earlier) with microisolation caging. Participants (n = 30) were blinded to the purpose of the study. Participants were provided PPE (gloves, disposable gown, shoe covers, hair bonnet, and face mask) and asked to dress for entry into a rodent barrier room. Participants randomly selected a task card and then entered and performed the task written on the card. A thin layer (1 measured ounce) of contamination powder was applied to an area directly inside of the animal room door in a 2.5- × 2-ft rectangle (drawn on the floor). All participants were observed to ensure that they walked through the powder as they entered the room (Figure 2 A and B). Upon exit from the animal room, all PPE was discarded in a trash receptacle directly outside of the animal room, and participants were given an informational sheet explaining the nature of the experiment and what was being evaluated. After participants were finished, the room was examined with a black light for distribution of powder in all areas, including the hood, all areas of the rack, inside and outside of the cages, inanimate objects within the cages, room floor, room walls, and entry door. The experiment was performed with the inclusion of shoe covers (Krypton Powder, green), after which the room was sanitized to remove all traces of powder. The experiment then repeated without shoe covers (XR7, blue).

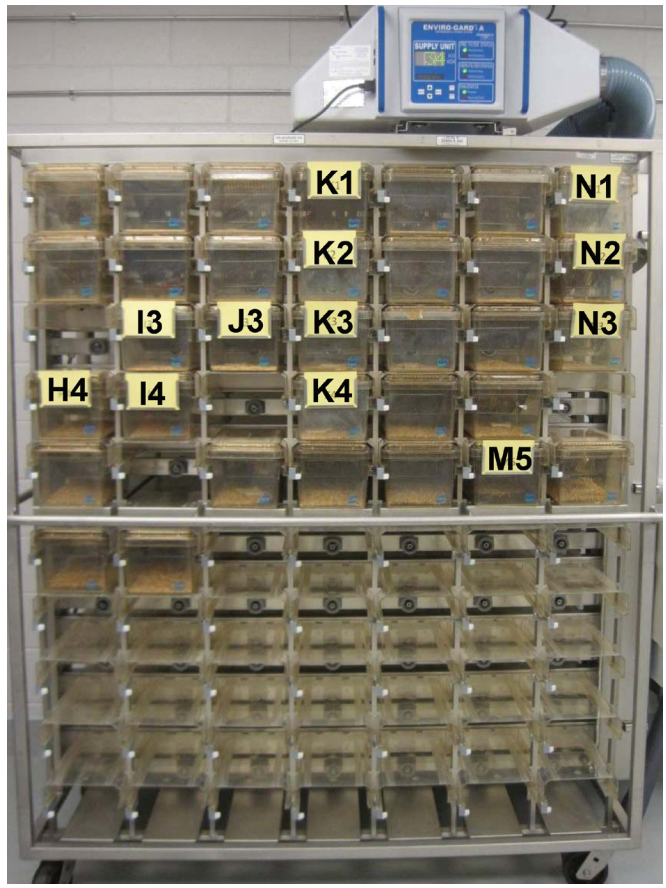

Figure 1. (A) Ventilated mouse rack with cage locations marked for assigned tasks.

Powders of different colors were used to avoid cross contamination and contradicting results between experimental runs. This method is a pass–fail test that requires a dark room and a black light. The limit of detection is based solely on visual inspection and works on any material with a contaminant that fluoresces under black light, provided that the material examined does not fluoresce itself. The limit for detection of contamination powders is unpublished. However, we easily detected 0.01 g under black light during preliminary experiments, in which decreasing concentrations of powder were spread on paper.

Study 2: Contamination from the floor to personnel. An empty animal room on an unused hallway with entry and exit doors on opposite sides of the room was selected for use. Participants were blinded to the purpose of the study. A PPE station was set up in the hallway directly outside of the animal room, and all views into the animal room were obstructed. Large sections of 2.5- × 2-ft rectangle white paper were taped to the ground in front of the PPE station, to limit the spread of the contamination

Figure 2. (A) Krypton and XR7 contamination powder. (B) A thin layer (1 oz) of XR7 powder applied to the floor inside of the animal room door. Powders appear white in UV light.

powder and make it more difficult for participants to see the powder. One ounce of powder was applied in a thin, uniform layer to the top of the paper. Inside the room, black lights were set up to view any spread of the contamination powder on participants. Participants (n = 30) entered the hallway one at a time and were instructed to don PPE, either with or without shoe covers, while standing on the white paper. In our experience, it is common practice among animal facilities for personnel to don shoe covers while standing in the hallway rather than as the threshold is crossed into the animal room. The order in which PPE was put on and the amount of time required to put do so were recorded. Participants then were instructed to enter the animal room, where they were examined using a black light. Photographs were taken of any fluorescence. Participants exited the room through the opposite doorway, to avoid interfering with the next participant.

Study 3: Contamination from outside into the vivarium. The distance from entry points (outside) of buildings to the entry door to the animal vivarium was measured. Contamination powder was applied directly to the floor, participants (n = 30) walked through the powder for a predetermined distance, and the floor then was examined for fluorescence under black light. The type and tread depth of shoes worn by participants walking through the powder were noted.

Footwear. We randomly surveyed 35 animal facilities employees to determine the most common types of shoes worn within the vivarium. Shoe tread depth was measured on all employees surveyed.

Statistics. All data are presented as mean ± SEM. Data analysis was performed by using GraphPad Instat software (GraphPad Software, San Diego, CA).

Results

Study 1: Contamination from the floor to the rack or cage. Examination of the animal room floor indicated no discernable difference in the spread of contamination powder in normal traffic patterns based on whether shoe covers were worn (Figure 3 A and B). None of the cages or inanimate objects within cages retained contamination powder after normal animal room tasks were completed (Figure 4 A). Contamination powder identified one participant who used the step stool and another who used the lower rung of the ventilated rack as a step to gain access to the top row of cages (Figure 4 B and C).

Study 2: Contamination from the floor to personnel. Participants were asked to put on 5 pieces of PPE (gown, bonnet, face mask, gloves, and shoe covers). Most personnel put on the gown first (47%), face mask second (47%), hair bonnet third (27%), and shoe covers fourth (53%). All participants put gloves on last (Figure 5). Black light examination showed that all participants who were asked to don shoe covers had fluorescence in multiple areas, most notably on gloves and gowns, especially the sleeve and cuff area (Figure 6 A). No fluorescence was noted on participants who did not apply shoe covers (Figure 6 B). The amount of time to don PPE was 78 ± 3 s with shoe covers compared with 50 ± 2 s ($P < 0.0001$, n = 30) without shoe covers.

Study 3: Contamination from outside into the vivarium. The entry door to the multispecies vivarium was 166 ft from the outside entrance to the building. When participants walked through contamination powder placed on the floor, powder was detected by black light illumination only for 32.3 ft, less than 20% of the distance from the outside door to the entry door of the vivarium. The average distance from the outside into any animal facility at our institution was 122 ± 18 ft^2 (n = 12), with the shortest distance being 40 ft^2. The average distance from any vivarium entrance into the nearest rodent room was 72 ± 12 ft^2 (n = 12), with the shortest distance being 36 ft^2.

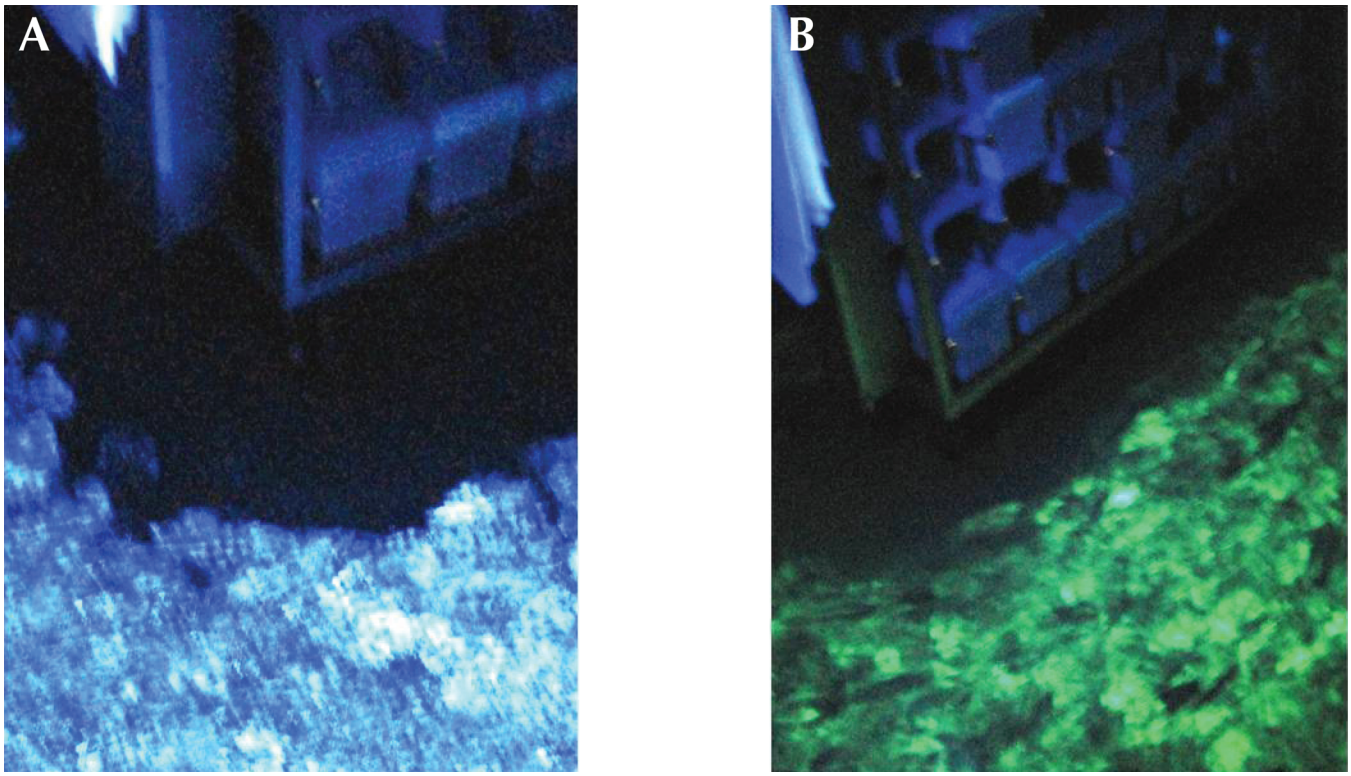

Figure 3. Black-light examination of the floor after the completion of study 1. Floors were examined after each participant, representative picture shown demonstrating the traffic pattern in either the (A) absence (XR7, blue) or (B) presence (Krypton, green) of shoe covers.

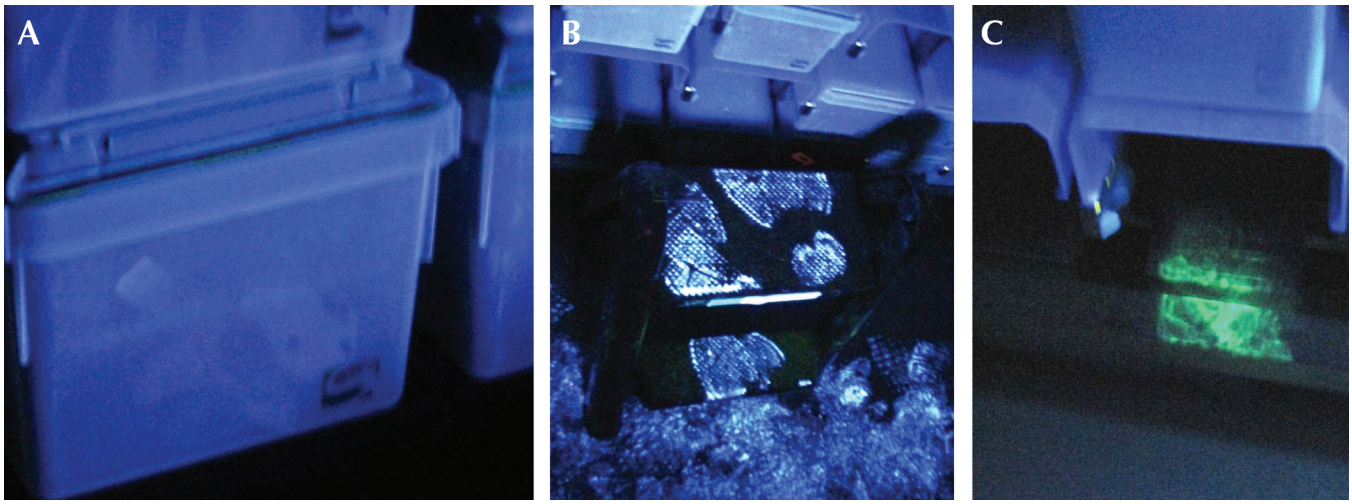

Figure 4. Black-light examination of animal room equipment after completion of study 1. (A) No contamination powder within any of the cages or on any of the inanimate objects within cages (representative cage side that was handled during the study shown). (B) XR7 powder (indicating a participant without shoe covers) on a step ladder used to access the top row of cages. (C) Krypton powder (indicating a participant with shoe covers) on the lower rung of the ventilated rack, which was used as a step to gain access to the top row of cages.

Footwear. Tennis shoes were the most common types of shoes worn by personnel on a daily basis (19 of 35, or 54% of employees surveyed). The most common tread depth of shoes worn for work was less than 0.5 cm (Figure 7).

Discussion

PPE is thought to keep both animals and humans safe in the laboratory animal setting. The general consensus within the laboratory animal community is that PPE is important; however, why it is used and which PPE is necessary remains an active topic of discussion. At the 2010 ACLAM forum, the use of shoe covers was a clear topic of interest. Results from the ACLAM forum survey indicated that 49% of respondents felt that disposable shoe covers represented the most effective method to prevent contamination of infectious agents into the animal facility;[2] however, little research addresses this premise. Although a recent publication indicates that the use of disinfectant mats or disposable shoe covers may reduce the bacterial load on rodent room floors,[1] the implication of decreased bacteria on the floor for rodents maintained in microisolation or ventilated caging is unclear. In addition, 64% of ACLAM survey respondents identified that rodents were housed in microisolation cages

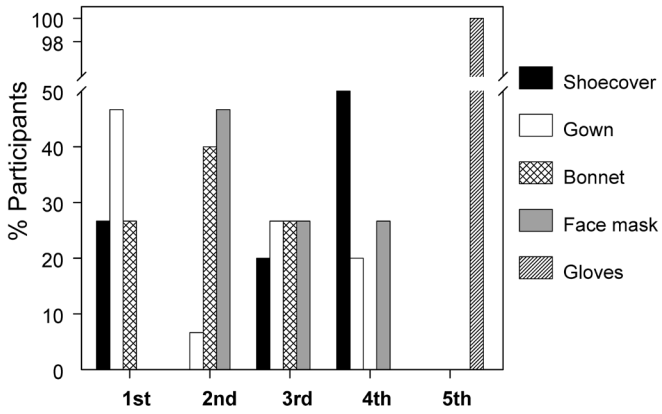

Figure 5. Participants (n = 30) were asked to put on 5 pieces of PPE (gown, bonnet, face mask, gloves, and shoe covers). The order in which participants put on the PPE items is expressed as a percentage for each item.

or barrier facilities, and only 13% used open cages without filter tops.[2] Microisolation caging and individually ventilated rodent caging systems have been shown to be very effective at controlling pathogen transmission between cages within the same room, and even on the same rack, for a variety of disease outbreaks.[4,5,9] The increased use of microisolation housing units warrants re-evaluation of the PPE necessary to maintain rodent health status.

The University Laboratory Animal Resources division at The Ohio State University consists of 150,542 ft^2 of vivarium space (including procedure rooms, cage wash, and storage), with 12 active vivaria and 176 animal rooms. Standard PPE required for entry into rodent barrier rooms has included disposable gown, shoe covers, face mask, hair bonnet, and gloves (Figure 8). All barrier rodent work is required to be performed within a biosafety cabinet, and approximately 99% of all rodents are housed in ventilated microisolation cages. When working in the biosafety cabinet and changing rodent cages, animal facilities personnel are required to change gloves between sets of cages belonging to different investigators. Rodent room floors are cleaned daily and sanitized twice weekly by using NPD one-step cleaner (contact time, 10 min; Steris, Mentor, OH), and any rodents that reach the level of the floor are required to be quarantined or removed permanently.

The institution employs 50 animal care staff, 4 animal care supervisors, and 15 veterinary medical care staff who access the vivarium daily. In all, there are 1749 animal users (including the animal facilities staff) with the potential to access one or more vivaria. On average, rooms with a card-swipe entry are accessed 33 times each day, with increased usage occurring in late spring and early summer, coinciding with the approval of research grants and incoming graduate students. Several factors were deemed important in the consideration for the use of shoe covers: contamination of animals from organisms on the floor; personnel safety (animal care and investigator staff); and contamination of research animals with pathogens acquired from outside the vivarium. The studies reported here were undertaken in an attempt to determine the effect that shoe covers have on 2 of these points: contamination of animals from organisms on the floor and contamination of research animals with pathogens acquired from outside.

We chose 2 contamination-tracking powders, in light of their availability, ease of use, and appropriate size. Both of these powders (XR7 [blue] and Krypton Powder [green]) are clearly visible under black light illumination, but both powders are difficult to detect when applied to a surface (Figure 2). Initial studies were performed to determine whether contaminants on the floor reach the level of the cage. Contaminant powder placed just inside the door on the animal room floor allowed participants to track through the powder on their way into the room. Each participant selected a task at random and was required to complete that task prior to exiting the room. All applicable biosafety cabinet and disinfectant usage was observed when cages were opened or 'animals' handled. Although the contamination powders are considered nontoxic, inanimate objects were used to represent live animals during this study. According to the manufacturers' specifications, contamination powder is approximately 44 μm in diameter and extremely light (powder was observed to float during application to the floor). However, no powder was detected within or on any rodent cage when observed under black-light illumination (Figure 4 A). Footprints were detected on the floor throughout the room in normal traffic patterns (Figure 3), on the step stool, and (in one instance) on the bottom rung of the rack, suggesting the usefulness of these compounds for employee training purposes (Figure 4). There was no visually detectable difference in the amount or distribution of powder in the presence or absence of shoe covers, indicating that none of the participants touched the floor or their feet once the shoe covers were in place and participants were in the room.

In the second study, contamination powder was placed on a white square of paper (so that the powder was virtually invisible), and participants were asked to stand on the paper and put on PPE either with or without shoe covers. Most people put on the disposable gown first and shoe covers fourth; all participants donned gloves last (Figure 5). Only those participants that put on shoe covers had contamination powder on their gloves and other PPE, indicating that the act of donning shoe covers provided significant opportunity for contaminants to be picked up off the floor and potentially come in contact with caging, equipment, or animals (Figure 6). The shoe covers used for these studies were nonstatic polypropylene with nonskid bottoms. Different types of shoe cover materials (polyethylene or latex) might alter the results of this study.

Finally, contamination powder was placed on the floor, and participants were asked to wear their own shoes and walk through the powder, in an attempt to determine how far contaminants picked up on shoes outside the facility are carried along the floor. The longest distance at which contamination powder could be detected was 32 ft^2. The shortest distance from outside any building to the vivarium door was 40 ft^2, with an additional distance of 36 ft^2 being required to reach the closest rodent room door. Tennis shoes were the most common shoe type worn by animal care staff, and although 17% of the personnel surveyed wore shoes with a tread depth exceeding 1 cm, tread depth did not affect how far the contamination powder traveled on shoe bottoms. These data indicate that items picked up outside are unlikely to remain on shoes all the way into the vivarium, under standard conditions.

Several considerations should be addressed regarding the control of infections transmitted through the environment including food, water, direct contact, air, and fomites. Direct, pathogen-related issues primarily involve virulence, contact time, and hardiness within the environment. Most of the research involving the dynamics of infection transmission has been performed by evaluating human and livestock populations.[7,11] Contamination powder used for these studies was very large size (44 μm) in comparison to most pathogens. Aerosol transmission of disease has been suggested to require

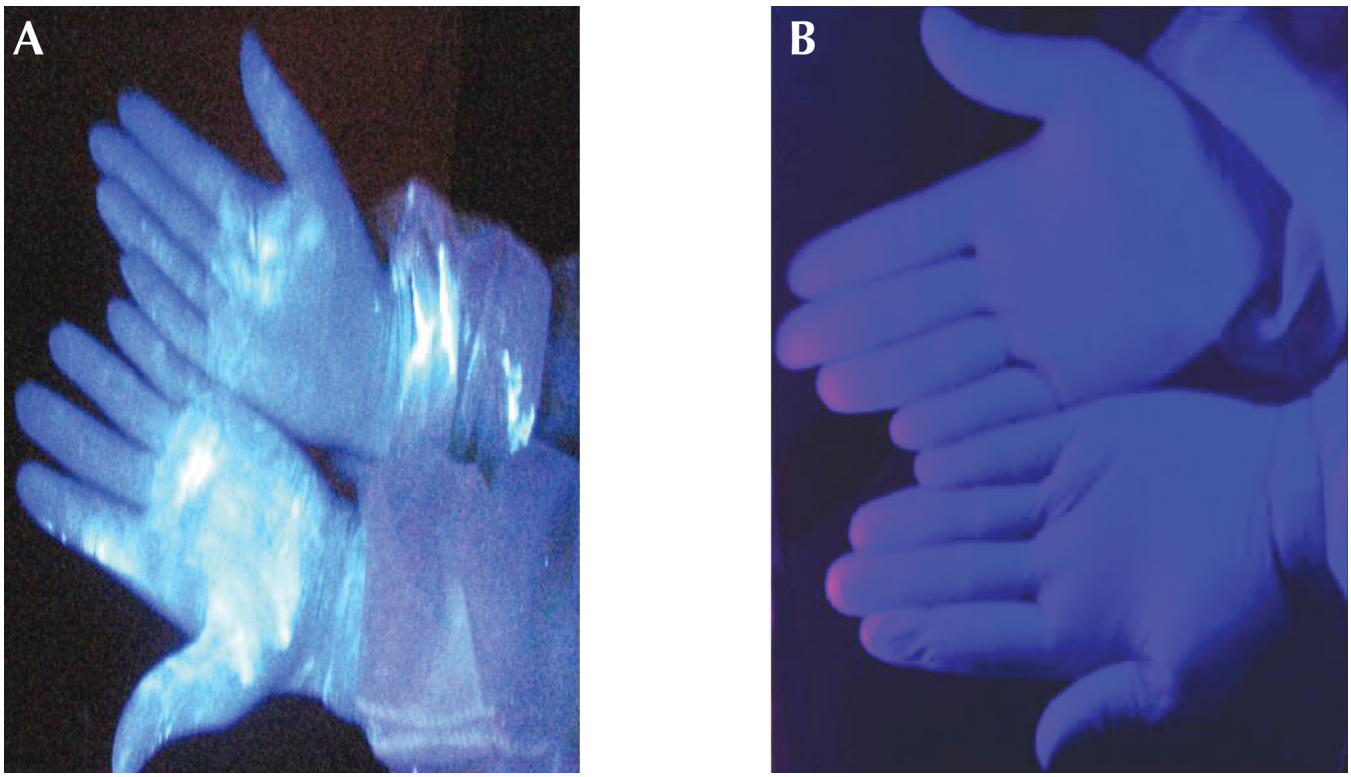

Figure 6. Black-light examination of participants in study 2 immediately after donning of PPE. (A) XR7 powder fluorescence on gloves and cuffs of gowns when shoe covers were applied. (B) Absence of fluorescence on gloves and cuffs when shoe covers were not applied (n = 30 for each group).

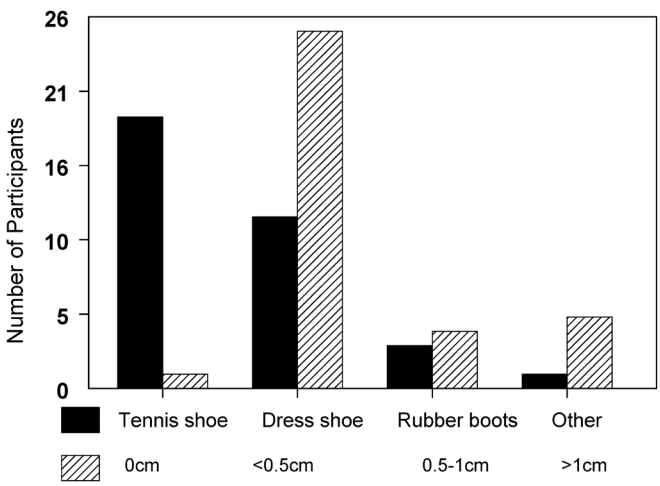

Figure 7. The recorded number of personnel (n = 35) wearing the described shoes and the different tread depths of these shoes.

a particle size of 10 µm or smaller.[6] The average size of bacteria is approximately 2 µm. However, particle size is not a primary consideration in the determination of transmission rates via fomites (direct contact).[11] This information supports the acceptability of contamination powders for tracking transmission from the floor through direct contact.

Although not a primary consideration for the implementation of these studies, cost savings is an important factor in the consideration of PPE requirements. The institution currently provides all of the PPE for animal room entry (Figure 8). From July 2009 through June 2010, $16,079 was spent on shoe covers, an amount that was second only to that for disposable gowns ($44,343). Hair bonnets ($3297), face masks ($7959), and disposable gloves ($6708) were purchased also, making the total spent on PPE for the fiscal year $78,369 (Figure 9). Therefore, shoe covers accounted for 20% of the total budget spent on PPE for the fiscal year of 2009. At the individual level, this amount represents a 17% PPE cost savings for each person donning full PPE (bonnet, mask, gloves, gown, and shoe covers). Our facility typically does not require that personnel change their shoe covers when traveling from one rodent room to another within the same facility; however, personnel are required to adhere strictly to room entry order and always to move from cleanest to dirtiest room according to assigned room entry order. Notably, 45% of ACLAM survey respondents indicated that shoe covers had to be changed between rodent rooms within the same facility,[2] a practice that likely greatly increased shoe cover usage and thus cost. Practices regarding the type of shoe cover used and the frequency of changing them vary dramatically between different institutions. The economic effect of eliminating shoe covers from general usage needs to be examined for each individual institution.

Time should be factored into discussions about cost. Participants were timed as they donned standard PPE (with and without shoe covers) before entering barrier rodent rooms. The addition of shoe covers added 28 s to the amount of time required to put on PPE. As part of the current study, an informal survey of 30 care staff revealed that the average staff member changes PPE 6 times (5.9 ± 0.6 times) during the course of the workday. When calculated by using ANOVA, this small increase in time was found to be 'extremely significant' ($P < 0.0001$) and represents a time savings of 13 h per year per person. The cost of this time savings equates to a yearly savings of approximately $7669 based on the average hourly pay rate of $12.29 for 48 husbandry staff members. This dollar amount does not take into account higher-paid employees, such as facility supervisors and

Figure 8. (A) PPE required for rodent barrier room entry at our institution includes hair bonnet, face mask, disposable gown, gloves, and shoe covers.

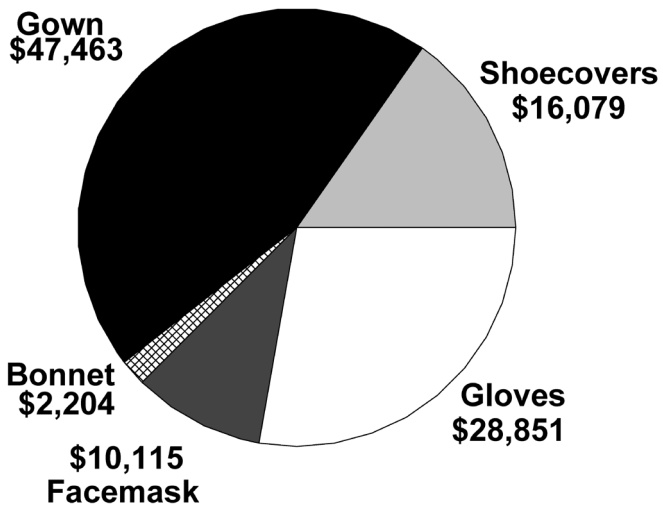

Figure 9. Total amount spent on shoe covers in relation to other PPE items from July 2009 through June 2010.

veterinary care personnel. Discussion of the number of times that staff are required to change shoe covers also emphasizes the ergonomic issues associated with the bending and twisting necessary to change footwear. PPE typically is donned while personnel stand in the hallways immediately outside of the animal room. A recent report from the National Institute for Occupational Safety and Health indicates that repetitive bending and twisting of the body as part of normal workplace activities is common and that this type of biomechanical stress causes increased risk for musculoskeletal disorders, such as back pain.[20]

Accepted policies and guidelines concerning aseptic surgery and hospital sanitation for human medicine are used frequently in the veterinary and laboratory animal medicine arena. In 2000, the fourth Operating Room (OR) Manager survey for human hospitals identified the use of shoe covers as one of the "sacred cows—rituals blessed by time that don't necessarily improve patient outcomes."[14] This survey was sent to 255 hospital subscribers and 94 ambulatory surgery centers. Among the 243 respondents, 86% reported that shoe covers were optional, and those that did use them primarily were concerned with keeping shoes clean from blood and body fluid splashes. Literature from the 1990s indicates shoe covers are ineffectual for decreasing the risk of surgical site infections,[10,21] and the 1999 Centers for Disease Control guideline specifies that shoe covers have never been shown to decrease infection risk or to lower bacterial counts on operating room floors and that they should never be worn for this purpose.[13] Finally, a more recent review of the literature from 1950 to 2003 found no evidence to support the effectiveness of shoe covers for controlling infection from microorganisms on the floor to open wounds or surgical sites on patients.[17]

After careful examination of the literature and results presented here, the veterinary staff at our institution determined that shoe covers were not necessary to protect against the spread of excluded pathogens within rodent rooms and that their use actually offers a potential for contamination of personnel from contact with shoe bottoms. The Ohio State University laboratory animal resources division maintains close contact with its principal investigators through an advisory group that is composed of faculty animal users from 8 of the colleges within the university. In Spring 2010, this advisory group and the IACUC approved the recommendation to stop the distribution and use of shoe covers within rodent rooms. Notification that shoe covers would no longer be required within rodent rooms was sent by email from the Director of University Laboratory Animal Resources to animal users, and laminated signs were posted at the entrance to animal room doors next to the PPE cart detailing the results of these studies.

The current studies indicate that the application of shoe covers provides a source of contamination for gloves that may outweigh the benefit of their use. Based on risk analysis, the costs of shoe covers and personnel time, and the lack of benefit to the rodent barrier containment system, the discontinuation of shoe covers seemed an obvious conclusion. Shoe covers may still be warranted in specific situations, and standard operating procedures concerning the order in which PPE items are applied should be considered if the use of shoe covers is deemed necessary.

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