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Extending the Use of Disposable Caging Based on Results of Microbiologic Surface Testing

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Prions are proteinaceous infectious agents that are highly resistant to denaturation. Sterilization of prion-contaminated mouse cages requires chemical agents and increased autoclave temperatures that damage traditional cages, thus increasing facility costs. Disposable cages are a possible alternative that might decrease replacement costs without compromising the environment of the mice. We compared our standard protocol of changing traditional cages and bedding once every 2 wk to an experimental protocol using disposable cages in which only the bedding was changed once every 2 wk over an 8-wk period. We hypothesized that disposable cages would retain an acceptable level of cleanliness (measured by ATP swabs and contact plates) for at least 8 wk when bedding is replaced every 14 d. Results from ATP swabs and contact plates showed no difference between the 2 protocols during the 8-wk experiment. Prolonged use (that is, as long as 8 wk) of disposable cages had no additional environmental concerns, compared with traditional cages.

Abbreviations: CB, cage bottom; CS, cage side; RLU, relative light units; RODAC, replicate organism detection and counting; CT, wire cage top

Sanitation and sterilization of animal caging is essential to minimize contamination of personnel and cross-contamination between experiments and vivaria. Sanitation is regularly performed by using commercial cage-wash equipment. Steam sterilization is the most widely used and dependable sterilization technique[2] and is routinely performed at our facility at 121 °C for 15 min. However, prions (proteinaceous infectious agents) are not inactivated by standard sterilization processes.[9] The recommended sterilization process to inactivate prions is to autoclave cages at 134 °C for 90 min.[1,9,10] In our experience, this increased temperature and time result in cracks and discoloration in traditional cages after a single sterilization cycle, potentially decreasing the cage lifespan from 2 to 3 y to 2 wk. Therefore, we sought to find a cost-effective alternative caging for housing our prion-infected mice.

Disposable cages are plastic, recyclable cages that are typically discarded after each cage change and are an alternative to traditional, nondisposable cages when autoclaving is required. Disposable cages replacement can become costly when the cages are replaced at every cage change, whereas reusing disposable caging by replacing the dirty bedding at regular intervals might reduce their cost. In addition, their reuse could decrease the carbon footprint of the facility.

According to the *Guide for the Care and Use of Laboratory Animals*, "There is no absolute minimal frequency of bedding changes; the choice is a matter of professional judgment and consultation between the investigator and animal care personnel."[5] The cage-changing interval depends on the number and size of animals, fecal and urinary outputs, wetness of bedding, primary enclosure size, and experimental conditions.[5] A previous study showed that ammonia concentrations do not exceed levels that result in adverse effects in mice after 17 d without changing the bedding, but cages were considered dirty by staff at 14 d due to excessive amounts of feces and soiled bedding.[8] Therefore, an alternative option to extending the cage-changing frequency is to change the soiled bedding according to our standard 2-wk cage-changing protocol without discarding the disposable cages for multiple bedding changes.

ATP-based monitoring and replicate organism detection and counting (RODAC) plates are commonly used in animal facilities, food production facilities, hospitals, and drug companies to test sanitation protocols.[1,11,12] ATP-based monitoring devices use bioluminescence to detect live or dead organic material on surfaces.[12] RODAC plates are commonly used in conjunction with ATP-based monitoring because RODAC plates detect cultivatable, aerobic organisms, whereas ATP-based methods do not distinguish between dead and live organic material.[12]

Here we used ATP swabs and RODAC plates to compare the current 2-wk cage-changing protocol to the disposable caging protocol where the soiled bedding was changed every 2 wk without replacing the cage components for a total of 8 wk. All cages were individually ventilated and contained noninfected, healthy mice. We hypothesized that the microbiologic environment would not differ significantly between the 2 groups and that disposable cage bottoms could be reused multiple times before disposal.

Materials and Methods

Animals. The experiment used 41 mice (age, 2 to 6 mo) of various strains (B6.129s4-Ccr2^{tm1Ifc}/J, C129S4-(B6)-Ccr2 ^{tm1Ifc}, and B6.129-Rage^{tml}); 15 male and 26 female mice were randomly assigned according to sex and strain into traditional cages ($n = 7$) and disposable cages ($n = 34$). Cages contained 1 to 5 mice. Mice were free of prions, Sendai virus, mouse hepatitis virus, minute mouse virus, mouse parvovirus, Theiler murine encephalitis virus, rotavirus, *Mycoplasma pulmonis*, pinworms, and ectoparasites. Mice were housed with unrestricted access to chow (Teklad Irradiated Diet 2918, Harlan Laboratories, Madison, WI) and filter-sterilized water before and during the experiment. Mice were maintained on a 14:10-h light:dark cycle at a temperature

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of 21 to 24 °C. All experimental procedures were approved by the IACUC and conducted at an AAALAC-accredited facility.

Cage materials. The 7 mice housed in traditional caging were separated into 3 cages (catalog no. CG09B01Small Mouse II Cage, Thoren Caging Systems, Hazleton, PA; $3064.00 per 200 units [all costs are given in USD]). The disposable-caging group comprised 37 mice allocated into 10 disposable cage bottoms (catalog no. CG09B09 Small Mouse II cage, APET Disposable, Thoren Caging Systems; $450.00 per 100 units). Both protocols used the same wire cage cover (catalog no. CC01B01 1B to hold 16-oz water bottle and feed, Thoren Caging Systems; $15,720.00 per 500 units) and filter cover (catalog no. FCo1DHN Filter Cover, Thoren Caging Systems; $3084.00 per 600 units) and were placed on a ventilated rack. Each cage contained 50 g (400 mL) autoclaved aspen bedding (catalog no. 7093 Teklad Shredded Aspen Bedding, Envigo, Indianapolis, IN) and 2 autoclaved napkins as enrichment.

We compared the costs of sterilizing traditional cages according to both standard and prion protocols with those of disposable cages discarded after 2, 4, 6, 8, and 10 wk. The lifespan of traditional cages autoclaved by using the standard sterilization protocol was 2 y (104 wk). The lifespan of traditional cages autoclaved by using the prion sterilization protocol was 2 wk. Wire cage covers, filter tops, water, food, enrichment, and bedding were not included in the cost analysis because they were the same between groups. Labor costs to load and unload the autoclave for a single cycle, which sterilizes 40 traditional cages, was calculated to be $15.75, or $0.39 per cage. The utility cost to run the autoclave was not included in the comparison.

Cage-changing protocols. All cage-changing and sampling procedures were performed in an animal transfer station (Maxi-Miser Change Station, Thoren Caging Systems) by personnel wearing appropriate personal protective equipment. Traditional cages were changed according to our standard protocol: cages were changed every 2 wk, and wire tops were changed every 4 wk. For disposable cages, the bedding was replaced every 2 wk, and the same disposable cage and wire top were used for the entire 8 wk. For both protocols, water bottles were changed weekly and filter tops changed every 4 wk.

Microbiologic sampling. Microbiologic sampling was completed by using ATP swabs (AccuPoint Advanced Sampler, Surface, Neogen, Lansing, MI) and RODAC plates (BBL RODAC Plate, Becton and Dickinson, Sparks, MD). Three ATP samples and 3 RODAC plate samples were collected from each cage at each time point. Samples were collected at baseline (0 wk) and 1, 2, 4, 6, and 8 wk. To delineate the sample area, 10-cm^2 rectangles were drawn onto the outside of each cage by using a wax pencil prior to the initiation of the experiment (Figure 1).

Sample collections were performed during cage changes, except at the 1-wk time point. Prior to sampling, ATP swabs and RODAC plates were removed from storage (4 °C) and warmed to room temperature for 30 min. During cage changes, mice that were housed in traditional cages were directly placed into a new cage containing 50 g aspen bedding. Mice housed in disposable cages were placed in a temporary holding cage and then returned to the disposable cage after sampling was completed. Soiled bedding from disposable cages was placed in a biohazard bag for decontamination. Any bedding remaining in the sample area was removed by wiping a sterile napkin over the area. ATP samples were performed by swabbing across the entire rectangle (Figure 1). RODAC plates were firmly pressed onto the sampling area for 10 s. Disposable cages were refilled with 50 g autoclaved aspen bedding and mice returned to the cage. ATP swabs were immediately read automatically (AccuPoint2 Advanced Reader, Neogen).

Figure 1. Sampling locations (10 cm^2 rectangles) were drawn onto the outside of each cage by using a wax pencil. Samples were obtained from the cage side (pink and yellow boxes), cage floor (dark and light blue boxes), and underside of the wire top (orange and red boxes). A, ATP swabs; R, RODAC plates.

RODAC plates were incubated for 72 h at 35 °C, and bacterial colonies were counted. RODAC plate results were graded according to our current sanitation protocol: excellent, 0 to 5 cfu; good, 6 to 15 cfu; fair, 16 to 25 cfu; and poor, 26 cfu or more. Plates containing colonies that were too numerous to count were notated as 90 colonies for calculations. ATP results were graded according to our current sanitation protocol: samples with 0 to 150 relative light units (RLU) have passing results and are considered clean; samples that read between 151 to 300 RLU are marginally clean; and samples that score 301 RLU or more have failing results.

Statistical analysis. Statistical analysis was done by using SAS 9.4 (SAS Institute, Cary, NC. A 2-sample *t* test was performed to determine the difference between the mean number of mice for the traditional and disposable cages. Traditional and disposable cages were compared for each location (bottom, side, and top), test (ATP or RODAC), and time (0, 1, 2, 4, 6, and 8 wk) by using the Wilcoxon rank-sum test. Comparisons were adjusted for multiple testing (by location and test) by using Benjamini–Hochberg tests. A *P* value less than 0.05 was considered statistically significant.

Results

ATP swab results. ATP results (Tables 1 through 3) were measured in RLU. ATP averages did not differ significantly between the traditional and disposable cages at any time point throughout the experiment. The average RLU from wire tops (CT), cage bottoms (CB), and cage sides (CS) did not differ significantly between traditional and disposable cages. No samples collected from CT scored above 150 RLU, whereas scores of 4 samples from CS and 6 samples from CB exceeded 300 RLU. Each of the 4 CS samples that exceeded 300 RLU was from different disposable cages at various time points (cages 5 and 6 at the 2-wk time point, 7 and 8 at 4 wk). In addition, 5 of the 6 CB samples that exceeded 300 RLU were from disposable cages at various time points (cage 3 at the 6-wk time point, cage 7 at 4 and 8 wk, cage 8 at 8 wk, and cage 9 at 4 wk), and the CB sample from traditional cage 1 at the 4-wk time point scored above

Table 1. Individual and average ATP and RODAC plate results for samples collected from wire tops

	No. of mice	Baseline (0 wk)		1 wk		2 wk		4 wk		6 wk		8 wk	
		ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC
Disposable cages													
1	3	0	4	0	0	0	17	0	90	60	0	0	0
2	4	0	90	13	0	0	0	0	0	79	0	0	0
3	3	0	0	0	0	0	0	2	0	9	0	23	0
4	5	0	0	0	0	0	4	0	0	28	0	88	0
5	4	0	90	0	0	0	0	21	0	0	0	5	0
6	3	0	0	0	0	0	0	46	2	0	0	0	0
7	5	0	0	4	0	0	0	40	1	0	0	8	0
8	1	0	0	15	0	0	0	0	0	0	0	0	0
9	3	0	0	12	0	0	0	55	0	1	0	0	0
10	2	0	0	16	0	0	0	0	0	0	0	0	0
Mean ± 1 SD	3.4	0 ± 0	18 ± 38	6 ± 7	0 ± 0	0 ± 0	2 ± 5	16 ± 22	9 ± 28	18 ± 29	0 ± 0	12 ± 28	0 ± 0
Traditional cages													
1	3	0	0	0	0	0	0	79	0	21	0	0	0
2	1	0	2	5	90	0	0	8	0	0	0	0	0
3	3	0	0	0	0	0	0	7	0	14	0	0	0
Mean ± 1 SD	2.3	0 ± 0	0.7 ± 1	2 ± 3	30 ± 52	0 ± 0	0 ± 0	31 ± 41	0 ± 0	12 ± 11	0 ± 0	0 ± 0	0 ± 0

ATP results are recorded as relative light units (RLU); RODAC plate results are recorded as number of colonies counted after incubation at 35 °C for 72 h.

Table 2. Individual and average ATP and RODAC plate results for samples collected from cage sides

	No. of mice	Baseline (0 wk)		1 wk		2 wk		4 wk		6 wk		8 wk	
		ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC
Disposable cages													
1	3	0	0	0	0	0	1	0	0	0	0	0	0
2	4	0	0	0	0	0	0	0	0	0	0	0	0
3	3	0	0	7	2	0	0	0	0	1	0	0	0
4	5	0	0	0	0	0	4	117	0	0	0	0	0
5	4	0	0	0	0	415	0	0	2	182	0	67	0
6	3	0	0	0	0	302	0	0	0	48	0	12	0
7	5	0	0	3	0	0	0	425	0	0	0	1	0
8	1	0	0	191	0	0	0	552	0	0	0	0	0
9	3	0	0	0	0	245	0	4	0	0	0	0	0
10	2	0	0	0	0	0	1	2	0	15	0	21	0
Mean ± 1 SD	3.4	0 ± 0	0 ± 0	20 ± 60	0.2 ± 0.6	96 ± 160	0.2 ± 0.4	110 ± 205	0.2 ± 0.6	25 ± 57	0 ± 0	10 ± 21	0 ± 0
Traditional cages													
1	3	0	0	0	0	168	11	5	0	0	0	28	4
2	1	0	0	18	0	0	0	5	0	0	0	0	0
3	3	0	0	0	4	0	0	6	0	5	0	9	0
Mean ± 1 SD	2.3	0 ± 0	0 ± 0	6 ± 10	1 ± 2	56 ± 97	4 ± 6	5 ± 0.6	0 ± 0	2 ± 3	0 ± 0	12 ± 14	1 ± 2

ATP results are recorded as relative light units (RLU); RODAC plate results are recorded as number of colonies counted after incubation at 35 °C for 72 h.

300 RLU. Other than the CB of cage 7 at 4 and 8 wk, individual cages did not have consistently elevated RLU. Cages that had ATP results of greater than 301 RLU did not have failing RODAC results at the same time point or location.

RODAC plate results. RODAC plate results (Tables 1 through 3) were recorded as the number of colonies per plate. Colony counts did not differ significantly between disposable cages and traditional cages at any time point or between CB, CS, and CT from disposable or traditional cages.

Seven samples at various time points and from different cages, cage designs (that is, traditional and disposable), and sampling areas yielded numbers of colonies that reflected poor sanitation (that is, more than 25 colonies). A total of 6 RODAC plate samples contained colonies that were too numerous to count: 4 samples from CT (disposable cage 1 at the 4-wk time point, disposable cages 2 and 5 at baseline, and traditional cage 2 at 1 wk) and 2 samples from CB (disposable cage 2 at the 4-wk time point and traditional cage 1 at 8 wk). The colonies on the RODAC plates for the 2 CT samples with colonies too numerous to count at baseline differed from all other colonies seen during the study; these plates were completely covered with fungal overgrowth and no individual colonies could be distinguished. In addition, these 2 cages had 0 cfu at every time point after baseline. The RODAC plate sample collected

Table 3. Individual and average ATP and RODAC plate results for samples collected from cage bottoms

		Baseline (0 wk)		1 wk		2 wk		4 wk		6 wk		8 wk	
	No. of mice	ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC	ATP	RODAC
Disposable cages													
1	3	0	0	0	0	0	30	0	0	0	0	0	0
2	4	0	0	0	0	0	0	0	90	29	0	0	0
3	3	0	0	7	0	0	5	0	0	663	1	0	0
4	5	0	0	0	0	0	1	0	0	16	0	198	0
5	4	0	0	0	0	0	0	123	4	0	0	0	0
6	3	0	10	0	0	0	0	217	8	6	0	4	0
7	5	0	0	49	0	0	0	681	12	0	0	517	0
8	1	0	0	11	0	0	4	70	1	38	0	730	0
9	3	0	0	4	0	0	0	757	2	0	2	0	0
10	2	0	0	4	0	0	0	8	1	0	0	0	0
Mean ± 1 SD	3.4	0 ± 0	1 ± 3	7 ± 15	0 ± 0	0 ± 0	4 ± 9	186 ± 290	12 ± 28	75 ± 207	0.3 ± 0.7	145 ± 264	0 ± 0
Traditional cages													
1	3	0	0	1	0	251	0	517	0	0	0	86	90
2	1	0	0	0	0	131	0	6	0	34	1	0	5
3	3	0	0	0	0	0	0	147	0	8	5	0	0
Mean ± 1 SD	2.3	0 ± 0	0 ± 0	0.3 ± 0.6	0 ± 0	127 ± 126	0 ± 0	223 ± 264	0 ± 0	14 ± 18	2 ± 3	29 ± 50	32 ± 51

ATP results are recorded as relative light units (RLU); RODAC plate results are recorded as number of colonies counted after incubation at 35 °C for 72 h.

at the 2-wk time point from the CB of disposable cage 1 had 30 colonies.

No cages yielded more than 25 colonies at multiple time points or sampling locations at the same time point. Cages with RODAC samples that produced more than 25 colonies did not have failing ATP results at the same time point or location.

Housing density. Uninfected mice were housed at various housing densities to assess the effect of the number of mice housed in a cage on the cage microbiologic environment, to assess whether the maximal cage density of 5 mice resulted in high ATP values and colony counts. Regardless of cage design, the microbiologic environment in the cage did not differ over time, according to results of samples from CT, CS, CB over time ($P = 0.23$ to 1.0). In addition, the microbiologic environment did not differ with cage density ($P = 0.17$). No animal morbidities or behavioral changes were observed in either group during the study period.

Cost analysis. The cost per cage per week was compared between traditional cages and disposable cages was completed (Table 4) to evaluate the cost difference between these protocols. Due to the dramatic decrease in lifespan, traditional cages used in prion research have a 15-fold increase in cost compared with traditional cages autoclaved by using the standard sterilization protocol. Disposable cages discarded after 2 wk cost $2.25 per cage per week, whereas traditional cages cost $7.66 per cage per week when discarded after 2 wk due to autoclave damage. Extending the use of disposable caging from 2 wk ($2.25) to 8 wk ($0.56) saves $1.69 per cage per week. Disposable caging could result in an additional savings of $0.09 per cage per week over traditional caging during a 104-wk lifespan if the use of disposable caging were extended to 10 wk ($0.45).

Discussion

ATP tests and RODAC plates are effective at determining the success of sanitation procedures[3] and are commonly used in animal facilities for this purpose. RODAC plates measure cultivable bacteria, whereas ATP-based tests indirectly measure a mixture of biologic forms. We used both tests in this study to compare the microbiologic environment of traditional cages that were changed according our standard 2-wk cage-changing protocol with disposable cages in which the bedding was replaced every 2 wk, thus extending the use of the disposable cages to 8 wk. The ATP and RODAC plate results showed that, compared with traditional cages, extending the duration of use of disposable cages without altering the cage-changing frequency has no to minimal effect on the cage microbiologic environment for as long as 8 wk.

According to our facility's current sanitation protocol, ATP samples yielding 301 RLU or more and RODAC plates with more than 25 colonies have failing results. Although specific cages at various locations and time points had failing results, no cages failed both ATP and RODAC plate tests at any single time point, no cages failed either test at multiple time points, and the number of failing results did not increase over the progression of the experiment. Had both the ATP and RODAC plate tests failed at any single time point or if the number of failing results had increased over time, we would have required replacement of the disposable caging.

The 4-wk time point had higher ATP averages than the remaining time points, perhaps because different personnel collected data at different time points. Because residual bedding had to be removed prior to sampling, one aspect of sampling that could not be standardized was the force used to wipe the cage prior to sample collection. One person might have wiped more vigorously than another, thus removing more microbes and resulting in lower values. Because an autoclaved dry napkin was used to wipe away remaining bedding between bedding changes, this step should be incorporated into the disposable cage-changing protocol if other facilities adopt this protocol to extend the lifespan of disposable caging.

The ATP and RODAC results did not correlate at every time point. This decreased correlation could be because the mouse microbiome consists primarily of anaerobes and fastidious organisms, such as Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae, and Ruminococcaceae.[4] These organisms would be measured during ATP monitoring but would be

Table 4. Cost comparison of traditional cages autoclaved by using the standard sterilization protocol with a lifespan of 104 wk and of traditional cages autoclaved by using prion sterilization protocol with a lifespan of 2 wk with disposable cages discarded after 2, 4, 6, 8, or 10 wk

Cage Type	Lifespan (wk)	Cost ($/cage/wk)
Traditional:		
	104	0.54
	2	8.05
Disposable:		
	2	2.25
	4	1.13
	6	0.75
	8	0.56
	10	0.45

Cost comparison includes cost of caging and labor for autoclaving but does not include autoclave utilities, wire cage covers, filter tops, water, food, enrichment, or bedding.

unlikely to grow on RODAC plates, which select for aerobic organisms. The limitations of RODAC plates in detecting anaerobes and fastidious organisms can result in a falsely low result and should be considered according to each facility's needs. In addition, ATP-based tests can underestimate levels of bacteria due to decreased lysis of pure cultures of gram-negative bacteria.[12] However, this drawback is less problematic with mixed populations of bacteria, as seen in feces, and was not expected to play a significant role in this study.

The use of disposable caging is cost-effective compared with traditional caging discarded after a single cage change due to the increased temperatures and pressures required for prion decontamination. Although the last time point in this study was 8 wk, there was no significant difference from the standard cage-changing protocol at this time point, and it therefore can be assumed that the disposable cage bottom could have been used for as long as 10 wk as well. Extending the use of disposable caging to 10 wk is almost an 18-fold decrease in cost compared with traditional caging discarded after 2 wk and a 5-fold decrease in cost compared with disposable caging discarded after 2 wk. It is important to note that the cost comparison did not include the utilities to run the autoclave, and including these in the comparison would create even greater savings when using disposable caging. The results of the cost comparison in addition to the microbiologic environment data show that extending the use of disposable caging has no effect on the microbiologic environment and decreases facility cage-replacement costs. Furthermore, the mice showed no health or behavioral changes. Since implementing the reuse of disposable caging, we have not noted a change in our quarterly sentinel monitoring results.

Prions are resistant to many standard sterilization techniques.[1,7,9,10] Guidelines recommend the use of single-use disposable equipment when in contact with prions.[6] Cages used at our facility can be damaged after a single sterilization using the high temperature, high-pressure autoclave cycles for prion decontamination, reducing their lifespan and therefore decreasing their cost effectiveness. Disposable cages meet the recommended guidelines for using single-use equipment and save on replacement costs. The current study demonstrates the lack of effect on the microbiologic environment, compared with standard protocol, when the use of disposable cages is extended by changing the bedding every 2 wk. Extending the use of disposable cages could save facilities replacement costs and decrease total waste production and does not need to be limited to prion studies.

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