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Evaluation of Various IVC Systems According to Mouse Reproductive Performance and Husbandry and Environmental Parameters

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IVC systems are marketed for improving the health and management of mouse colonies. The current study compared mouse reproductive performance and husbandry and environmental parameters among 3 high-density (HD) IVC rack systems (RS1, RS2, and RS3), which were present in separate but comparable rooms. Three breeding trios each of Swiss Webster (CFW) and BALB/c mice were placed in each rack (*n* = 36 female, *n* = 18 male). Reproductive indices were measured for 3 breeding cycles over 2 generations; indices included time to parturition, litter size and pup weight, survivability, and interbirth interval. Over 18 wk, personnel used scoring systems to evaluate each RS daily to every other week according to cage dirtiness, need for spot changing, ease of cage changing, daily health checks, and cage wash processing. Macroenvironmental parameters (temperature, relative humidity, noise, total particulate matter) were measured weekly over 14 wks. Microenvironmental parameters (temperature, relative humidity, NH_3, CO_2, O_2) of 2 cages each of male and female CFW mice (4 mice/cage) on each RS were measured at 6 time points over 2 wks. RS1 had significantly smaller mean litter sizes of CFW mice (mean ± 1 SD, 6.5 ± 2.9 pups) as compared with both RS2 (9.5 ± 1.7 pups) and RS3 (9.3 ± 3.8 pups). RS1 scored as being significantly easier to process through the cage wash. RS2 had significantly lower room noise levels (46.0 ± 5.0 dBA) but higher humidity (58.6% ± 8.9%) as compared with both RS1 (43.7% ± 9.9%) and RS3 (46.0% ± 12.0%) over the 2-wk cycle, particularly at 8 and 12 d after cage change. In conclusion, in terms of mouse reproductive performance and husbandry and environmental parameters, each system had at least 1 advantage over the other 2. Therefore, various factors should be considered when choosing an IVC system for mice.

Abbreviations: CCS, cage-changing station; HD, high density; PM, particle mass; RH, relative humidity; RS, rack system; TPM, total particulate matter

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Mice are the most frequently used models for both human and animal research and are the species of choice for most biomedical research.[1,22] According to the *Guide for the Care and Use of Laboratory Animals*, "appropriate animal housing and management are essential contributors to animal well-being, the quality of animal research and production, teaching or testing programs involving animals, and the health and safety of personnel."[23] Many caging options are available for mice in research settings, and these vary greatly in their intended purposes and design, ranging from static microisolators to IVC. In particular, IVC systems are becoming increasingly common in research vivaria that house mice,[1,4,12,41,53] because these systems improve husbandry standards, promote biocontainment, reduce potential exposure of mice to pathogens that may confound research goals, and may reduce the release of particulates and allergenic contaminants that are produced by mice and their bedding.[2,33,42,43,54]

IVC often differ in ventilation strategies, structural design, and housing capacity.[26,32,41] IVC ventilation can function through either positive- or negative-pressure.[27] Negative-pressure ventilation is an effective method of reducing allergen exposure in personnel and of biocontainment, especially if mice are administered biohazards.[20,34,39] On the other hand, to reduce risk of exposure of mice to airborne pathogens as for maintaining SPF colonies, running IVC in positive-pressure mode is often advisable.[15] Nonsealed IVC may not facilitate allergen reduction when pathogen exclusion efforts require positive-pressure ventilation.[15] To overcome this drawback, IVC are often designed with sealed lids that promote containment and allow them to be run in either positive- or negative-pressure ventilation modes.[15] In addition, some IVC rack systems (RS) are designed to house higher densities of cages than standard RS and are thus referred to as high-density (HD) RS. HD RS therefore save facility space and increase the total capacity of mice that can be housed in a given space. Although current literature does not define the caging density required for a RS to be considered HD, the HD RS that we evaluated in the current study contained 96 to 100 cages per rack. HD RS manufacturers achieve these increased cage densities through a variety of different structural manipulations, including rack alterations (for example, taller racks, less distance between the bottom row and the floor, denser spacing of rows or columns, dynamic carousal design), cage alterations (for example, shorter cages), and sometimes smaller rack footprints.

Previous studies have suggested that IVC RS may influence mouse reproductive performance and husbandry and environmental parameters,[1,2,10,11,19,21,35,36,40,41,52] but no studies to date

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have concurrently evaluated all these parameters in HD RS. The objectives of our present study were to compare mouse reproductive performance, personnel performance and evaluations, and environmental parameters among 3 different HD RS. To investigate any significant differences between HD RS, we conducted a comprehensive systematic analysis over a period of 8 mo. To determine any differences in reproductive performance, we bred 2 generations of 1 strain and 1 stock of mice that are known to differ in reproductive performance. Personnel using these RS performed a variety of novel evaluation scoring systems to assess HD RS. Finally, to identify any environmental differences, we monitored both the microenvironment and macroenvironment. We hypothesized that none of the parameters analyzed would differ among the 3 tested HD RS.

Materials and Methods

Ethical statement. All procedures involving animals were approved prior to implementation by the Johns Hopkins University Animal Care and Use Committee. All animals were housed in an AAALAC-accredited facility, and all procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*.[23]

Housing and husbandry. Mice were housed in 3 separate but comparable and adjacent rooms (41,751 to 41,760 sq ft), that each contained a different HD RS (RS1: NexGen MAX, Allentown Caging, Allentown, NJ; RS2: Optimice, Animal Care Systems, Centennial, CO; RS3, Emerald Rack, High Density EMM096X, Tecniplast, West Chester, PA). Each room was retrofitted with RS according to vendor recommendations, but only one rack per room was used for the purposes of this study. All rooms were maintained at temperatures of 67 to 77 °F (19.4 to 25.0 °C), relative humidity of 30% to 70%, and a 14:10-h light:dark cycle. All autoclaved cages had corncob bedding (Teklad 1/4″ Corncob Bedding, Envigo, Madison, WI) at a depth recommended by the RS manufacturer (approximately 1/4 in. for all systems) and a single cotton square (Nestlets, Ancare, Bellmore, NY) for enrichment. Bedding and cotton squares were replaced at each cage change, which was performed by husbandry staff at the end of every 2-wk period. To evaluate and account for any possible effects of the cage-changing stations (CCS) used (Phantom2 Animal Transfer Station, Allentown Caging; AniGARD II Animal Transfer Station, The Baker Company, Sanford, ME; Aria Changing Station, Tecniplast), CCS were rotated among rooms every other 2-wk cage-change period. Mice were provided ad libitum access to autoclaved feed (Teklad Global 18% Protein Extruded Rodent Diet, Envigo, Madison, WI) and fresh water via automatic watering systems. Air ventilation (RS1 and RS2, positive pressure ventilation, RS3, passive negative pressure ventilation) and cage air changes per hour (RS1, 55; RS2, 75; RS3, 29) were set to vendor recommendations.

Animals. The first generation of mice enrolled in this study (founders) consisted of 3 breeding trios each of Crl:CFW (CFW) and BALB/cAnNCrl mice (Charles River Laboratories, Kingston, NY), which were randomly assigned to each RS (age, 7 wk; n = 36 females, n = 18 males). The 6 cages per RS were strategically distributed on each rack such that cages were evenly separated from each other by at least 2 adjacent cage spaces to evaluate and account for any possible row or column effects. Once the offspring of the founder mice reached 7 wk of age, second-generation mice were selected randomly and formed into breeding trios to provide the same number of mice as for the founders (n = 36 females, n = 18 males). Colony mice were monitored for pathogens via serology and PCR analysis of soiled-bedding sentinel mice and by exhaust-air–duct PCR assay and were negative for the following pathogens: Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse minute virus, mouse parvovirus types 1 and 2, Theiler mouse encephalomyelitis virus, reovirus, epizootic diarrhea of infant mice, lymphocytic choriomeningitis virus, ectromelia virus, murine adenovirus, murine cytomegalovirus, *Mycoplasma pulmonis*, fur mites (*Myobia*, *Mycoptes*, *Radfordia*), and pinworms (*Aspiculuris* and *Syphacia* spp.).

Study design and experimental procedures. *Reproductive and clinical indices.* Reproductive indices of the breeding trios were measured for 3 breeding cycles for both the founder and the second generation in the 3 RS. Indices included time to parturition (that is, time to first litter after formation of the trio), litter size, pup weights (measured at time of weaning on postnatal day 21[P21]), survivability (% of litter that survived to P21), and interbirth intervals. Analyses also compared the 2 strains (CFW and BALB/c) by RS on interbirth intervals, litter size and pup weights, and survivability. The number and nature of clinical cases for each RS were documented.

Husbandry, cage wash, and researcher personnel evaluations. Figure 1 shows various scoring systems used by personnel. At least 2 personnel per category participated in the study to incorporate interpersonnel variability. Vendor representatives trained husbandry and cage-wash personnel in the use of each RS. Over 18 wks, 2 husbandry personnel used the scoring systems to evaluate RS every other week based on cage dirtiness and ease of cage changeout and daily based on ease of health checks. The time spent to change a single cage and frequency of spot changing were recorded also. The time spent to change a single cage was determined by recording the amount of time that husbandry personnel spent changing 7 cages, each of which contained 3 adult mice, for each RS at all time points except for the final time point, when the time to change 21 cages was recorded; total time spent was then divided by the number of cages changed at each time point to determine average amount of time spent per cage (that is, time spent to change a single cage). Every other week for 10 wk, 2 cagewash technicians used scoring systems to evaluate ease of cage processing on both the clean and dirty side of cage wash. Five research personnel used scoring systems to evaluate ease of use at least every other week. Two researchers evaluated RS for 10 wk, whereas 3 researchers evaluated them for only 2 to 4 wk due to COVID-19 governmental and institutional restrictions. For evaluations using scoring systems, lower numerical scores were assigned for better performance within each criterion (Figure 1).

Environmental monitoring. Macroenvironmental (room) parameters (temperature, relative humidity [RH], noise, total particulate matter [TPM], NH_3, CO_2, O_2) were measured at similar locations in each room (i.e., center and adjacent to cage-changing stations) between 0700 and 1800 h. Temperature and RH were measured by using the building's automated system (Metasys Building Automation System, Johnson Controls, Milwaukee, WI), which recorded data continuously. Quadruplicate measurements were taken concurrently with microenvironmental data sampling over a 2-wk cage change period on days 0, 5, 8, 11, 12, and 14 after cage change. Room noise levels were measured weekly over 14 wk as A-weighted decibels (i.e., the average of the minimum and maximum dBA at each time point) by using a sound meter (SM10 Sound Meter, Amprobe, Everett, WA; A-weighting, 30 to 130 dB; frequency range, 31.5 Hz to 8 kHz). Room ambient particle counts were measured weekly over 14 wk by using a portable particle counter (8000 Series Handheld Particle Counter, Particles Plus, Stoughton, MA). Room ambient gases (NH_3, CO_2, O_2) were measured at 6

Personnel	Evaluation	Criteria	Scoring System	Total Score Ranges
Husbandry	Cage dirtiness	- Fecal pellets - Urine spots - Feed on floor	0 = Soilage absent, clean bedding 1 = Mildly soiled, mostly dry bedding 2 = Moderately soiled, <1/4 bedding soiled 3 = Severely soiled, ≥1/4 bedding soiled	0 (cleanest)- 3 (dirtiest)
	Ease of cage change	1. Opening/closing cages 2. Working within hood 3. Stacking cages 4. Placing/handling feeders 5. Adding feed/enrichment 6. General cage handling	1 = Easy 2 = Neutral 3 = Difficult	6 (easiest)- 18 (most difficult)
	Ease of health checks	- Need to remove cages - Need for flashlight - Visibility of mice in cage	1= Easy 2= Neutral 3= Difficult	0 (easiest)- 3 (most difficult)
Cage wash technicians	Ease of cage processing: dirty side of cage wash	1. Cage disassembly 2. Stacking cages 3. Placing feeders 4. Bedding disposal	1= Easy 2= Neutral 3= Difficult	4 (easiest)- 12 (most difficult)
	Ease of cage processing: clean side of cage wash	1. Cage assembly 2. Placing bedding/feed/enrichment 3. Autoclaving	1= Easy 2= Neutral 3= Difficult	3 (easiest)- 9 (most difficult)
Researchers	Ease of Use	1. Accessibility of cage from rack 2. Accessibility of cage during use 3. Restraint of mice during use	1= Easy 2= Neutral 3= Difficult	3 (easiest)- 9 (most difficult)

Figure 1. Personnel evaluations. For evaluations with numerical criteria, total scores were calculated by adding scores for each criterion, with lower numerical scores being assigned for better performance within each criterion.

time points over a 2-wk cage-change period by using a portable gas monitor (MX6 iBrid Multigas Monitor, Industrial Scientific, Pittsburgh, PA) on days 0, 5, 8, 11, 12, and 14 after cage change.

Microenvironmental (cage) parameters (temperature, RH, NH_3, CO_2, O_2) of 2 cages each of male and female CFW mice (4 mice per cage) per RS were measured at 6 time points over a 2-wk cage-change period on days 0, 5, 8, 11, 12, and 14 after cage change (4 data samples per parameter per time point). Cage gases were measured by using the aforementioned gas monitor, with sampling tubing placed through the automatic watering system port of each cage. Cage temperature and RH were measured by using a portable thermohygrometer (model no. PMRH120, Compact Thermometer and Humidity Panel Meter, Cooper Atkins, Middlefield, CT) placed in the feeder of each cage.

Statistical analyses. Reported data are expressed throughout as mean values ± 1 SD. Statistical analyses were conducted by using Prism (version 8.4.3 for Windows, GraphPad Software, San Diego, CA). All data sets were assessed for normality (Gaussian distribution) both visually and through D'Agostino

and Pearson and Shapiro–Wilks tests. For parametric data, either 2-way or one-way ANOVA tests were performed, followed by Tukey multiple-comparison testing. For nonparametric data, Kruskal–Wallis or Friedman tests were conducted, followed by Dunn multiple-comparison testing. Linear regression analyses were conducted also. A P value less than 0.05 was considered statistically significant.

Results

Reproductive and clinical indices. Analyses of all reproductive and clinical indices revealed no significant differences between systems ($P > 0.05$ in all cases) when results were averaged (means) across both generations without separation based on strain or stock (Table 1). Similarly, analyses of all reproductive and clinical indices revealed no significant differences between systems (P > 0.05 in all cases) when results from the first and second generations were compared without separation by strain or stock.

Further analyses indicated few strain- or stock-dependent differences (Table 2). Litter sizes at weaning (postnatal day 21) were significantly smaller for BALB/c (5.8 ± 1.5) compared with CFW (8.4 ± 3.2) when mean values were not separated according to RS ($P < 0.0001$). CFW litter sizes at weaning were significantly smaller for RS1 (6.5 ± 2.9 pups) when compared with both RS2 (9.5 ± 1.7, $P = 0.007$) and RS3 (9.3 ± 3.8, P = 0.012). Although pup weights at weaning were significantly smaller for BALB/c (10.2 ± 1.3 g) compared with CFW (11.5 ± 1.9 g, $P = 0.0015$) when mean values were not separated according to RS, no significant differences were found when comparing pup weights for each strain or stock between RS. The percentage of pups surviving to weaning was significantly higher for BALB/c (96.1% ± 9.8%) as compared with CFW (80.5% ± 27.6%, $P = 0.0002$). The percentage of pups surviving to weaning did not differ between RS, but within RS1, the percentage of pups surviving to weaning was significantly higher for BALB/c (96.8% ± 9.5%) than CFW (70.6% ± 31.1%, $P = 0.008$).

The frequency of clinical calls did not differ significantly between systems. Although the total number of clinical calls reported within each RS varied numerically, the relatively few clinical calls within each RS did not produce any statistically significant differences. In total, 15 clinical problems were reported, including dystocia ($n = 7$), mastitis ($n = 1$), trauma (fight wounds, $n = 3$; lameness, $n = 1$), neoplasia (confirmed on diagnostic necropsy, $n = 1$; suspected, $n = 1$), and dehydration ($n = 1$). For litters, 48 clinical problems were reported, including maternal neglect ($n = 22$), cannibalism ($n = 18$), stillborn ($n = 2$), and undetermined causes of pup mortality ($n = 6$).

Husbandry, cage wash, and researcher personnel evaluations. Technician evaluations related to cage change yielded no significant differences between RS among all parameters analyzed (Table 3). Time spent to change a single cage did not differ between systems (all $P > 0.05$), but a significant effect was found over time (week) on time spent to change a single cage ($P = 0.03$). Time spent to change a single cage averaged across RS was significantly higher during week 2 (50.2 ± 3.3 s) as compared with weeks 8 (32.6 ± 0.5 s), 10 (34.5 ± 2.2 s), and 14 (29.6 ± 1.8 s, all $P < 0.05$; Figure 2). Furthermore, simple linear regression analysis revealed a significant negative relationship between week of cage change and time spent to change a single cage for all RS (all slopes were significantly nonzero; RS1, –1.6; RS2, –1.3; RS3, –1.4; all $P < 0.05$). Time spent to change a single cage did not differ between caretakers. Spot changing frequency (i.e., the average number of spot changes per 2-wk cage change period) was not significantly different between RS, but a significant effect of time (week) was found on the number of spot changes ($P = 0.041$). Simple linear regression analysis of spot change evaluations revealed that the slopes of RS1 and RS2 were significantly nonzero and negative (RS1 slope, –0.31; RS2 slope, –0.65; $P < 0.05$), indicating a negative relationship between the time and number of spot changes, whereas RS3 approached significance (slope, –0.05, $P = 0.169$). RS1 had significantly more spot changes during week 0 through 2 than during week 4 through 6, week 6 through 8, and week 8 through 10 ($P < 0.05$). Although personnel scores differed significantly from one another regarding ease of health checks, ease of cage change, and cage dirtiness (all $P < 0.001$), these parameters were not significantly different when mean scores were compared among RS.

Table 1. Reproductive indices (mean ± 1 SD) according to RS.

	RS1	RS2	RS3
Time to first litter (d)	25.4 ± 4.7	30 ± 11	28 ± 10
Interbirth interval (d)	38 ± 10	37.5 ± 9.3	39 ± 11
Litter weight at weaning (g)	75 ± 30	86 ± 30	85 ± 32
Litter size at P21 (# of pups)	5.8 ± 2.4	7.7 ± 2.6	7.6 ± 3.3
Pup weights at weaning (g)	12.2 ± 5.8	11.1 ± 1.9	10.6 ± 1.7
Pup survival (%)	87 ± 23	91 ± 14	95 ± 14
Clinical calls (%)	8 ± 14	13 ± 22	8 ± 14

Two generations of breeding trios were maintained for 3 breeding cycles per generation. There were no significant differences when comparing means between systems, regardless of stock or strain.
Pup survival = (no. of pups alive on postnatal day 21 / no. of pups alive on postnatal day 7) × 100%
Clinical calls = (average no. clinical calls / cage) × 100%

Table 2. Reproductive indices (mean ± 1 SD) averaged over both generations according to strain or stock.

	RS1		RS2		RS3		All systems	
	BALB/c	CFW	BALB/c	CFW	BALB/c	CFW	BALB/c	CFW
Interbirth interval (d)	38 ± 17	42 ± 10	32.8 ± 7.1	41 ± 12	41 ± 11	32.2 ± 6.9	37 ± 13	38 ± 11
No. of pups at weaning	5.4 ± 1.7	6.5 ± 2.9[a, b]	6.1 ± 1.8	9.5 ± 1.7[a]	5.86 ± 0.87	9.3 ± 3.8[b]	5.8 ± 1.5[c]	8.4 ± 3.2[c]
Pup weight at weaning (g)	9.9 ± 1.3	11.7 ± 1.5	10.3 ± 1.5	11.58 ± 2.02	10.3 ± 1.3	11.2 ± 2.3	10.2 ± 1.3[d]	11.5 ± 1.9[d]
Pup survival (%)	96.8 ± 9.5[e]	71 ± 31[e]	93.1 ± 13.2	87.2 ± 15.5	98.6 ± 4.9	84 ± 32	96.1 ± 9.8[f]	81 ± 28[f]

Two generations of breeding trios were maintained over 3 breeding cycles per generation. There were few significant differences between systems and strain or stock.
Pup survival = (no. of pups alive on postnatal day 21 / no. of pups alive on postnatal day 7) × 100%
[a]$P = 0.007$ when comparing mean CFW litter sizes at weaning for RS1 compared with RS2.
[b]$P = 0.01$ when comparing mean CFW litter sizes at weaning for RS1 compared with RS3.
[c]$P < 0.0001$ when comparing mean litter sizes averaged across all RS comparing between strain and stock.
[d]$P = 0.002$ when comparing mean pup weights at weaning averaged across all RS comparing between strain and stock.
[e]$P = 0.008$ when comparing mean percentage of pups surviving to weaning for CFW with BALB/c within RS1.
[f]$P = 0.0002$ when comparing mean percentage of pups surviving to weaning averaged across all RS comparing between strain and stock.

Table 3. Husbandry (cage change) evaluations.

	RS1	RS2	RS3
Time (s) to change 1 cage	36.4 ± 7.5	38.7 ± 7.1	35.6 ± 6.3
Ease of cage change	6.92 ± 0.38	9.00 ± 0.55	7.92 ± 1.02
Cage dirtiness	1.43 ± 0.42	1.54 ± 0.32	1.36 ± 0.28
Ease of health check	1.64 ± 0.37	1.79 ± 0.27	1.14 ± 0.24
Spot changes per week	0.50 ± 0.71	1.0 ± 1.4	0.10 ± 0.22

Data shown are shown a mean ± 1 SD. Each cage held 3 adult mice. Ease of cage change, cage dirtiness, and ease of health check evaluations are presented as the mean score values of the results of the scoring systems described in Figure 1. There were no significant differences (all $P > 0.05$) when comparing mean values between systems.

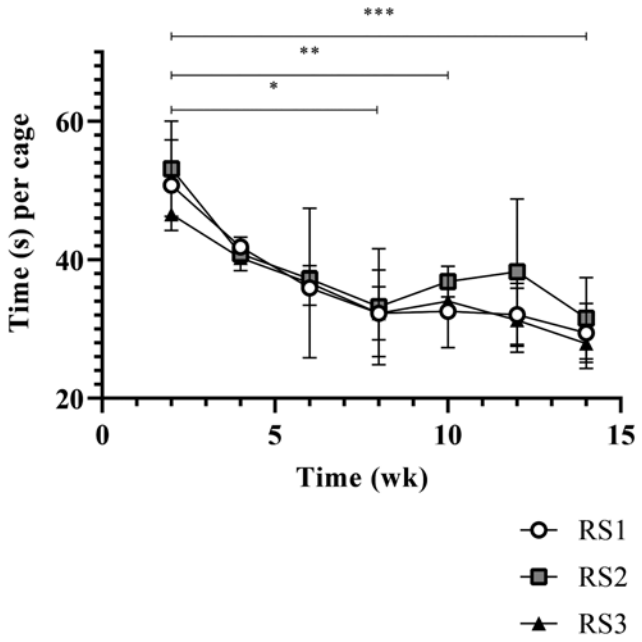

Figure 2. Time (s, mean ± 1 SD) to change a single cage containing 3 adult mice. There were no significant differences between RS. Across all RS,, means were significantly higher at week 2 (50.2 ± 3.3) as compared with weeks 8 (32.6 ± 0.5, *), 10 (34.5 ± 2.2, **), and 14 (29.6 ± 1.8, ***) (all $P < 0.05$).

Cage wash technician evaluations revealed few significant differences between RS (Table 4). System had a significant effect on total score evaluations for both the clean ($P = 0.002$) and dirty ($P = 0.007$) sides of cage wash. Although scores from 2 technicians were significantly different from one another for both clean ($P < 0.001$) and dirty ($P < 0.001$) sides of cage wash, their relative preference of RS was identical (i.e., RS1 > RS3 > RS2). Overall total scores for the clean side of cage wash were significantly lower for RS1 (3.0 ± 0.0) as compared with RS2 (8.0 ± 0.0; $P < 0.001$). At week 10 (final time point), total scores for the clean side of cage wash for RS1 (3.0) were significantly lower than RS3 (5.0) and RS2 (8.0), and RS3 (5.0) was significantly lower than RS2 (8.0; all $P < 0.001$). Likewise, total scores for the dirty side of cage wash were significantly lower for RS1 (4.0 ± 0.0) as compared with RS2 (11.17 ± 0.52, $P = 0.002$).

Researcher evaluations revealed no significant differences between RS (Table 4). Total scores for ease of use (including accessibility of cage from rack, accessibility of cage during use, and restraint of mice during use) were not significantly different between RS, with no significant differences over time (all $P > 0.05$).

Table 4. Cage wash technician and researcher evaluations.

	RS1	RS2	RS3
Cage wash technicians: Ease of cage processing			
Clean side of cage wash	3.0 ± 0.0[a]	8.0 ± 0.0[a]	5.4 ± 0.2
Dirty side of cage wash	4.0 ± 0.0[b]	11.2 ± 0.5[b]	10.0 ± 1.6
Researchers			
Ease of use	3.8 ± 0.4	5.5 ± 0.3	3.6 ± 0.7

Data are shown as mean score values ± 1 SD of the results of the scoring systems described in Figure 1.
[a]$P < 0.001$ when comparing evaluations of total scores of clean side of cage wash RS1 (3.0) was significantly lower than RS2 (8.0).
[b]$P = 0.002$ when comparing evaluations of total scores of dirty side of cage wash RS1 (4.0) was significantly lower than RS2 (11.17).

Analyses of RS mechanical problems and effects of CCS did not reveal any statistically significant differences. Frequency of reported mechanical problems were not significantly different between RS and included rack leak ($n = 1$ for RS1), broken feeder ($n = 1$ for RS1), cage leaks ($n = 4$ for RS2), and broken cage lid ($n = 1$ for RS2). No mechanical problems were reported for RS3 for the entire duration of the study. Statistical analyses of time spent to change a single cage, ease of cage change, litter sizes, and particle counts did not reveal any effect of CCS on any of these parameters (all $P > 0.05$; Figure 2).

Macroenvironmental monitoring. When compared over the 2-wk cage change period, room temperatures were not significantly different between rooms (Figure 3 A; RS1, 70.9 ± 1.6 °F; RS2, 71.6 ± 1.1 °F; RS3, 72.48 ± 0.88 °F; all $P > 0.05$). Significant differences were detected between RS at individual time points (days 0, 5, 8, 12, and 14, all $P < 0.05$). On day 0, the RS3 room was warmer (72.5 ± 0.3 °F) than the RS1 room (71.6 ± 0.3 °F). On day 5, RS2 was significantly warmer (70.3 ± 0.3 °F) than RS1 room (68.3 ± 0.4 °F), whereas the RS3 room (71.2 ± 0.0 °F) was warmer than both RS1 and RS2. On day 8, the RS2 room was warmer (70.1 ± 0.5 °F) than the RS1 room (69.5 ± 0.3 °F), whereas RS3 was significantly warmer (71.9 ± 0.3 °F) than both the RS1 and RS2 rooms. On day 11, there were no significant differences. On day 12, RS3 room (73.0 ± 0.1 °F) was significantly warmer than both RS1 (72.1 ± 0.1 °F) and RS2 (72.0 ± 0.0 °F) rooms. On day 14, the RS3 room (73.8 ± 0.2 °F) was significantly warmer than both the RS1 (72.1 ± 0.2 °F) and RS2 (73.0 ± 0.1 °F) room, and the RS2 room was warmer than RS1. Linear regression analysis revealed RS1 and RS2 slopes were not significantly nonzero (no relationship between days after cage change and temperature in room), but RS3 slope was significantly nonzero ($P = 0.005$) and positive (slope = 0.1), suggesting a positive relationship for RS3 room temperatures over time. However, the slopes of the lines were not significantly different from each other.

Room RH readings taken over a 2-wk cage change period (Figure 3 B) revealed no significant differences between rooms (RS1, 31.4% ± 14.3%; RS2, 29.9% ± 12.0%; RS3, 29.5% ± 12.6%; all $P > 0.05$). Simple linear regression analysis revealed all slopes were significantly nonzero and negative (all $P < 0.001$), but the slopes were not significantly different when compared between rooms ($P = 0.87$), with a pooled slope of –2.2. The room RH means for RS2 and RS3 fell below recommended limits; however, all average cage RH remained within acceptable limits.

Mean room noise level recordings (dBA, Figure 3 C) were significantly lower for RS2 (46.0 ± 5.0 dBA) than for both RS1 (53.1 ± 4.8 dBA, $P = 0.005$) and RS3 (54.0 ± 3.1 dBA, $P < 0.001$). In addition, the minimal noise level was lower for RS2 (40.2 ± 3.5 dBA) than for RS1 (47.7 ± 1.2 dBA, $P = 0.002$) and RS3 (51.6 ± 2.3 dBA, $P < 0.001$). Furthermore, maximal dBA was signifi-

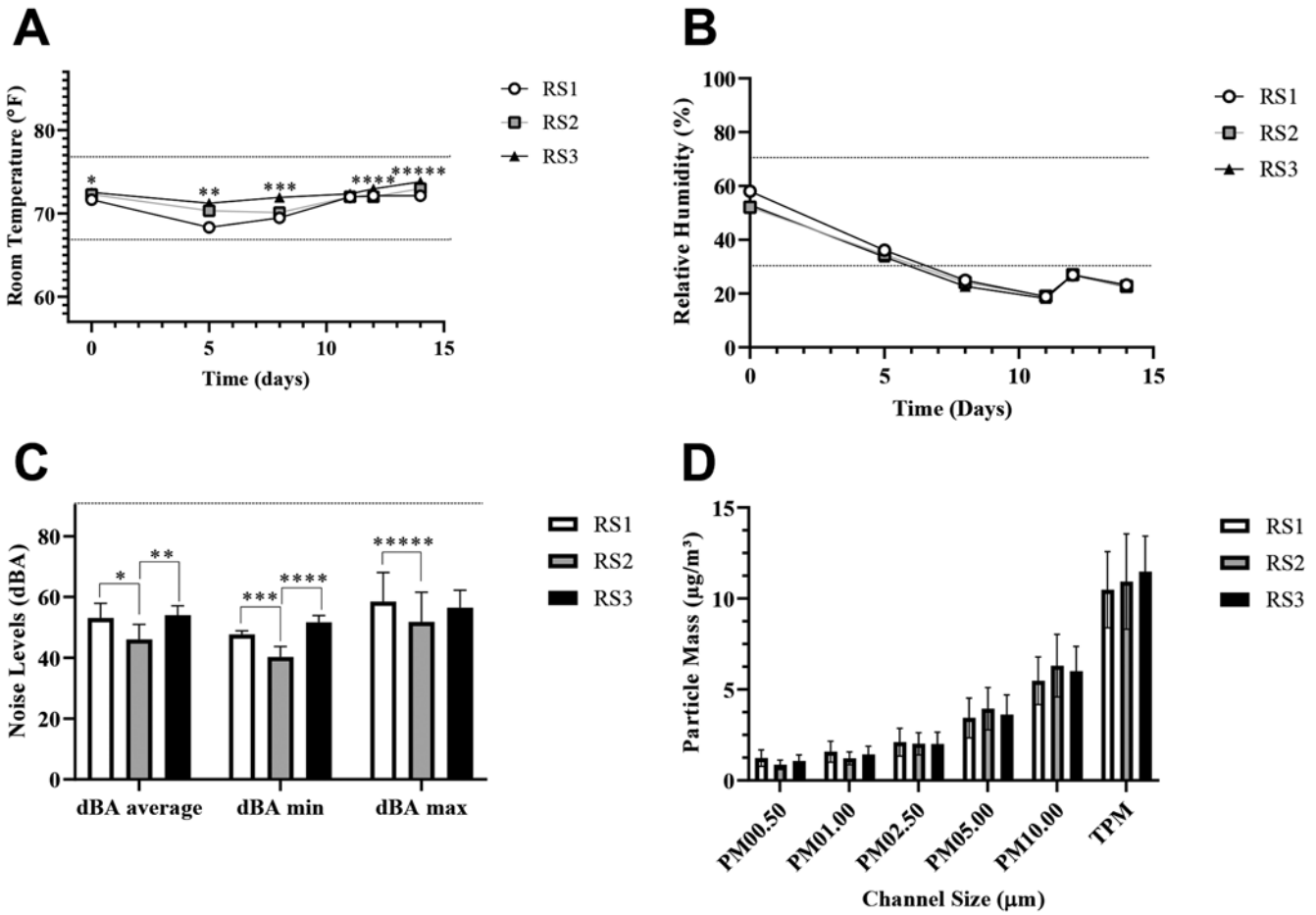

Figure 3. Room (macroenvironmental) parameters. (A) Room temperatures (°F, mean ± 1 SD) over a 2-wk cage change period. No significant differences between rooms when comparing overall mean values across 2-wk cage change period ($P > 0.05$). Significant differences were noted at individual time points between rooms ($P < 0.05$). On day 0, RS3 room was warmer than RS1 room. On day 5, RS2 was significantly warmer than RS1, and RS3 was warmer than both RS1 and RS2. On day 8, RS2 was warmer than RS1, and RS3 was warmer than both RS1 and RS2. On day 12, RS3 was warmer than both RS1 and RS2. On day 14, RS3 was warmer than both RS2 and RS1, and RS2 was warmer than RS1. Acceptable ranges posted for each room were 67 to 77 °F (dashed lines). (B) Room relative humidity (%, mean ± 1 SD). No significant differences when comparing overall means between rooms across 2-wk cage change period (all $P > 0.05$). Acceptable ranges posted for each room were 30% to 70% (dashed lines). (C) Room noise levels (dBA, mean ± 1 SD). Mean dBA average was significantly lower for RS2 than both RS1 and RS3. In addition, minimum dBA min was lower for RS2 than RS1 and RS3, and maximum dBA was lower for RS2 than RS1. All noise levels were under the recommended maximum exposure limit of 85 dB (dashed line).[23] (D) Room particle counts (mean ± 1 SD). No significant differences between RS when comparing all measured particle counts according to channel size (all $P > 0.05$).

cantly lower for RS2 (51.7 ± 9.8) when compared with RS1 (58.5 ± 9.6, $P = 0.009$).

Analyses of ambient air particles (particle mass [PM] channel sizes 0.50, 1.00, 2.50, 10.00 µm, and total particle mass [TPM], Figure 3 D) revealed no significant differences when comparing between systems (all $P > 0.05$). Additional analyses comparing particle counts on cage changing and noncage changing days revealed significantly higher values when averaged across all 3 systems on cage changing days (i.e., 14 µg/m^3) as compared with noncage changing days (9 µg/m^3; data not shown on Figure 3 D, $P < 0.001$). Evaluation of effects of CCS on particle counts revealed that mean TPM differed between CCS (i.e., 8.9 µg/m^3 compared with 13.10 µg/m^3; $P = 0.043$). No other significant differences in particle counts were found when comparing effects of CCS and of cage changing and noncage changing days.

All measured room gas values did not differ between systems. Mean room NH_3 levels over a 2-wk cage change period were not significantly different between systems and were all below 5 ppm (RS1, 4.8 ± 4.2 ppm; RS2, 4.7 ± 3.8 ppm; RS3, 3.2 ± 5.2 ppm, all $P > 0.05$). Mean room CO_2 levels over the 2-wk cage change period were not significantly different between systems and were all below 0.05% (RS1, 0.043% ± 0.022%; RS2, 0.035% ± 0.012%; RS3, 0.030% ± 0.019%; all $P > 0.05$). Mean room O_2 levels over the 2-wk cage change period were not significantly different between systems and were all approximately 21% (RS1, 20.97% ± 0.05%; RS2, 21.02% ± 0.04%; RS3, 20.98% ± 0.04%; all $P > 0.05$).

Microenvironmental monitoring. Mean cage temperatures over the 2-wk cage change period did not differ significantly between RS (RS1, 72.8 ± 1.9 °F; RS2, 74.9 ± 1.1 °F; RS3, 74.2 ± 1.1 °F; all $P > 0.05$; Figure 4 A) except at 2 individual time points (days 0 and 8). At day 0, RS2 cages (75.7 ± 1.3 °F) were significantly warmer than RS3 cages (72.7 ± 0.7 °F; $P = 0.023$). At day 8, RS1 cages (69.2 ± 0.76 °F) were significantly cooler as compared with both RS2 cages (74.4 ± 0.8 °F, $P < 0.001$) and RS3 cages (73.4 ± 1.4 °F, $P = 0.008$). No other significant differences were found when comparing between groups at each time point or over the 2-wk cage change period. Linear regression analysis revealed the slopes of the lines (cage temperature over time) were not significantly nonzero and were not significantly different. Mean room temperatures were not significantly different than cage temperatures for both RS1 and RS3 (all $P > 0.05$),

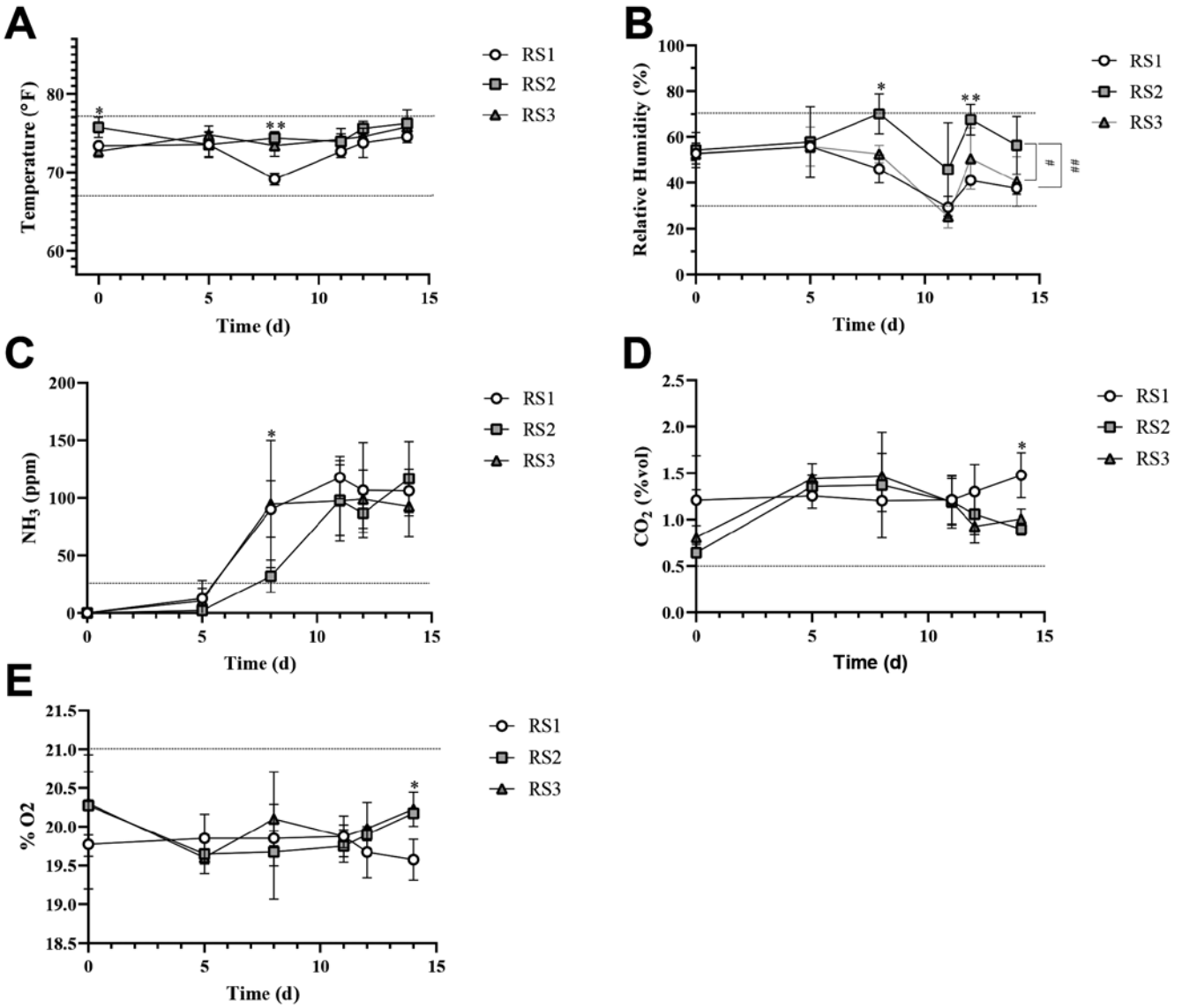

Figure 4. Cage (microenvironmental) parameters over a 2-wk cage change period. (A) Cage temperature (°F, mean ± 1 SD). Mean cage temperatures were not significantly different between RS over the 2-wk cage change period, except at 2 individual time points (days 0 and 8). At day 0, RS2 cages were warmer than RS3 cages ($P = 0.023$). At day 8, RS1 cages were cooler than both RS2 cages ($P < 0.001$) and RS3 cages ($P = 0.008$). Acceptable ranges posted for each room were 67 to 77 °F (dashed lines). (B) Cage relative humidity (%, mean ± 1 SD). Mean cage relative humidity over the 2-wk cage change period was higher ($P < 0.05$) for RS2 than both RS3 and RS1. At day 8, RS2 cages were more humid than both RS3 ($P = 0.044$) and RS1 cages (46.0 ± 6.2%, $P = 0.013$). At day 12, RS2 cages were more humid than RS1 cages. Acceptable ranges posted for each room were 30% to 70% (dashed lines). (C) Cage NH_3 (ppm, mean ± 1 SD). Mean cage NH_3 levels over a 2-wk cage change period were not significantly different between RS (all $P > 0.05$) except at day 8, when RS2 had significantly lower NH_3 levels than RS1. The dashed line indicates OSHA's recommended NH_3 exposure limits for humans (25 ppm) as an 8-h time-weighted exposure limit.[8] (D) Cage CO_2 (%, mean ± 1 SD). Mean cage CO_2 levels over the 2-wk cage change period were not significantly different between RS (all $P > 0.05$), except at day 14, when RS1 cages had significantly higher CO_2 levels than both RS2 ($P = 0.03$) and RS3 ($P = 0.049$). The dashed line indicates OSHA's permissible exposure limit of 5000 ppm by volume (0.5% concentration) as an 8-h time-weighted average.[46] Cage CO_2 levels exceeded 0.5% at all time points for all 3 RS. (E) Cage O_2 (%, mean ± 1 SD). Mean cage O_2 levels over the 2-wk cage change period were not significantly different between RS (all $P > 0.05$), except at day 14, when RS1 cages had significantly lower O_2 levels than both RS2 ($P = 0.03$) and RS3 ($P = 0.02$). Dashed line indicates normal atmospheric oxygen concentrations.[45]

but RS2 cages (74.9 °F) were significantly warmer than the RS2 room (70.7 °F, $P = 0.003$).

Mean cage RH over the 2-wk cage change period (Figure 4 B) were significantly higher for RS2 (58.6% ± 8.9%) as compared with both RS3 (46.2% ± 11.5%, $P = 0.04$) and RS1 (43.7% ± 9.9%, $P = 0.02$). At day 8, RH was higher in RS2 cages (70.0% ± 8.6%) than RS3 (52.5% ± 3.8%, $P = 0.044$) and RS1 cages (46.0% ± 6.2%, $P = 0.013$). At day 12, RH was higher in RS2 cages (67.5% ± 6.8%) than RS1 cages (41.0% ± 1.4%, $P = 0.007$). Linear regression analysis revealed that the slopes of lines (humidity over time) were significantly nonzero and negative for RS1 (slope, –1.5, $P < 0.001$) and RS3 (slope, –1.2, $P = 0.03$) but not for RS2 (slope, –0.2, $P = 0.8$); slopes were not significantly different between RS. No other significant differences were found when comparing between groups over the 2-wk cage change period and at each time point. Mean room RH was not significantly different than cage RH for any RS (all $P > 0.05$).

Mean cage NH_3 levels over a 2-wk cage change period (Figure 4 C) were not significantly different between RS (RS1, 72 ± 52 ppm; RS2, 56 ± 51 ppm; RS3, 66 ± 47 ppm; all $P > 0.05$), except

at day 8. At day 8, RS2 had significantly lower NH3 levels (32 ± 14 ppm) as compared with RS1 (90 ± 24 ppm; $P = 0.022$). Simple linear regression analysis revealed slopes of lines (NH_3 levels over time) were not significantly different between systems, but all slopes were significantly nonzero with a positive relationship between days after cage change and NH_3 levels (pooled slope, 8.9). NH_3 levels across all groups were significantly lower at day 0 and 5 as compared with days 8, 11, 12, and 14. All room NH_3 levels were significantly lower than respective cage NH_3 levels (all $P < 0.05$).

Mean cage CO_2 levels over the 2-wk cage change period (Figure 4 D) were not significantly different between RS (RS1, 1.3% ± 0.1%; RS2, 1.1% ± 0.3%; RS3, 1.1% ± 0.3%; all $P > 0.05$), except at the final time point (day 14). At day 14, RS1 cages had significantly higher CO_2 levels (1.5% ± 0.2%) than both RS2 (0.9% ± 0.1%, $P = 0.03$) and RS3 (1.0% ± 0.1%, $P = 0.049$). Simple linear regression analysis revealed that slopes of lines (CO_2 levels over time) were not significantly different between systems and were not significantly nonzero (that is, there was no relationship between time and CO_2 level). All room CO_2 levels were significantly lower than respective cage CO_2 levels (all $P < 0.05$).

Mean cage O_2 levels over the 2-wk cage change period (Figure 4 E) were not significantly different between RS (RS1, 19.8% ± 0.1%; RS2, 19.9% ± 0.3%; RS3, 20.0% ± 0.3%; all $P > 0.05$), except at the final time point (day 14). At day 14, RS1 cages had significantly lower O_2 levels (19.6% ± 0.3%) than both RS2 (20.2% ± 0.1%, $P = 0.03$) and RS3 (20.2% ± 0.2%, $P = 0.02$). Simple linear regression analysis revealed slopes of lines (O_2 levels over time) were not significantly different between systems and were not significantly nonzero (that is, there was no relationship between time and O_2 level). All room O_2 levels were significantly higher than respective cage O_2 levels (all $P < 0.05$).

Discussion

This study provides comprehensive analyses of 3 HD RS in regard to mouse reproductive performance, personnel evaluations, and environmental parameters. As a result of these analyses, this investigation provides an overview of important parameters that should be considered prior to investing in an HD RS and serves as a reference for any institution that intends to house mice for research purposes in an HD RS. The parameters evaluated in this study are important because appropriate animal housing is critical for animal health and welfare, research validity and reproducibility, and occupational health and safety. Furthermore, a major cost investment of any research vivarium is incurred from animal housing, such that any institution considering investing in an HD RS should consider its potential effects on the animals and husbandry, cage wash, and research personnel, to ensure an informed decision. When possible, independent evaluations and review of RS should be conducted in an objective and unbiased manner prior to investment.

Our findings suggest that mouse reproductive performance and quantity and nature of clinical cases did not differ between racks. As has been reported by the animal vendor and in previous studies,[16,41,52] strain and stock had effects on litter size, pup weights, and pup survival rates. We used outbred CFW and inbred BALB/c mice because they are commonly used in research and the reproductive performance of inbred strains is, in most cases, poor compared with that of outbred stocks,[50] thereby better capturing the variation in mouse reproductive performance commonly seen in vivaria. As expected, when mean values were not separated according to RS, mean litter sizes at weaning (postnatal day 21) and pup weights were significantly smaller for BALB/c than CFW. The mean percentage of pups surviving to weaning was significantly higher for BALB/c compared with CFW, perhaps reflecting the significantly smaller BALB/c litter sizes that allowed the dams to nurse pups adequately. Prior to their enrollment in this study, the animal vendor housed the founder mice in proprietary wire- and open-topped caging (not IVC systems). All mean litter sizes and pup weights produced during this study were consistent (within 1 SD or in the reported range) with the indices reported by the vendor, further indicating that mouse reproductive performance was not affected by the microenvironment or use of IVC systems. The CFW breeders had larger litters at time of weaning in the RS2 and RS3 cages when compared with the RS1 cages; this difference was likely due to a few large CFW litters in RS1 cages that were lost due to maternal neglect. Indeed, large litters are known to increase the risk of newborn mortality.[3] Within RS1 cages, survival rates were higher for BALB/c litters than CFW; this significant difference was not found in the other 2 RS. The few significant differences detected in reproductive indices were only observed when data were analyzed according to strain or stock. One limitation of the study was the use of only 1 strain and 1 stock of mice, especially given that most rodent vivaria house many, diverse mouse lines.

The number and nature of clinical cases were not significantly different between RS. Clinical cases were primarily reproductive in nature (e.g., dystocia) but few in number, indicating that overall animal health was not significantly nor differentially affected by the HD RS. Given that mice were used for breeding throughout this study, the predominance of reproductive-related clinical cases was not unexpected, and we suspect that this outcome would occur in other non-IVC cage systems housing comparable breeding colonies of mice.

Animal research and husbandry are demanding work. Animal housing equipment should be ergonomically appropriate and practical,[36] and the procedures implemented should be efficient and effective. The current study presents the first objective assessment of HD RS among various personnel using the equipment. A limitation of the personnel evaluations is their subjective nature, which we controlled for by having well-defined scoring criteria and at least 2 raters per category.

Husbandry (animal housing room) technician evaluations were not significantly different between RS among all parameters analyzed. The time spent to change a single cage significantly decreased over time for all RS, indicating improved efficiency in the cage changing process. This result indicated that personnel eventually became accustomed to using the RS and cages, in that the time spent to change a single cage was inversely proportional to the acclimation period. Although the technicians received instruction in working with IVC caging from the vendor of RS1 prior to the study, this instruction used a nonHD IVC RS and therefore did not skew the data for this study. At our institution (and as reported by others[14]), our husbandry staff typically change at least 200 cages daily. The time spent to change a single cage of a nonHD RS ranges from 40 to 144 s per cage, as compared with the final average of 30 s per cage of an HD RS for the 2 husbandry technicians enrolled in this study. Similarly, spot changing frequency was not significantly different between RS. In particular, RS1 had significantly more spot changes than other RS at the first biweekly period but not at subsequent time points, indicating that this difference was more likely an anomaly rather than a consistent association. We used a scoring system to simplify assessments of health checks, ease of cage change, and cage dirtiness, to achieve objectivity and consistency, but individual preferences likely contributed

to observed differences, particularly given that these differences were not maintained after averaging between the 2 husbandry technicians and comparing between RS. Therefore, our study reveals that although user preference may influence individual evaluations, differences in technician performance across racks systems were negligible.

Evaluations from cage wash technicians indicated that RS1 cages were easier to process through cage wash. Although both cage wash technicians had previous experience working with a nonHD IVC manufactured by the RS1 vendor and although cage washing equipment was designed by the vendor of RS1 (for nonHD IVC cages), both technicians indicated that their evaluations were not significantly influenced by this experience, given the different design of the HD RS used in this study. Furthermore, neither individual had prior work experience with any of the HD RS used in this study. Therefore, past work experiences are unlikely to have substantially influenced study results. Researcher evaluations indicated that all RS were easy to use for research purposes. These evaluations therefore suggest that the HD RS evaluated in this study were relatively comparable in their ease of use by researchers during their work with mice.

Incidence of mechanical issues like water leaks were not significantly different between RS. The leaks might have resulted from valve malfunction (although leaks are more commonly seen in old automatic watering systems) due to obstruction by either bedding or enrichment material. As reported by vendors, mechanical issues were rare. As mentioned, most mechanical issues develop in old equipment; therefore, long-term evaluation is needed to investigate RS, cage, and cage component durability. For example, plastic components such as degrading of cage bottoms and cracking of welded wire can only be assessed over time, after the cage components have been handled and are processed through cage wash repeatedly.

The RS used affected only a few macroenvironmental parameters. Although room temperatures for RS3 were significantly warmer at most individual time points, all mean room temperatures remained within the *Guide*-recommended room temperature (68 to 79 °F).[23] However, RS2 and RS3 mean room RH levels (RS2, 29.9% ± 12.0%; RS3, 29.5% ± 12.6%) fell below the *Guide*-recommendation of 30% to 70%, and all 3 rooms had RH levels below 30% for the last 4 time points.[23] However, clinical signs that could be attributed to warm temperatures (e.g., behavioral changes[51]) or low RH (e.g., ring tail) were not observed during this study.

When compared across systems, mean noise levels were significantly lower in the RS2 room, possibly because the racks did not rely on mechanical blowers and thus use passive negative pressure ventilation only. In contrast, RS1 and RS3 racks relied on blowers to achieve positive pressure ventilation. However, all rooms' noise levels were below the NIOSH recommended 8h exposure to minimize occupational induced hearing loss (85 dBA as an 8-h time-weighted average).[9] This noise level measurement was more relevant to occupational health and safety rather than to animal welfare because the sound meter frequency range was 31.5 Hz to 8 kHz and therefore it does not measure ultrasonic noise above 20 kHz, which is audible to mice.[44] Also, the meter measured dBA, which is relevant to human noise exposure but is not appropriate for estimating noise exposure in mice.[37,44]

Air quality did not differ significantly between rooms and was not compromised by the presence of the racks. Levels of ambient air particles were below the EPA primary (12.0 $\mu g/m^3$) and secondary (15.0 $\mu g/m^3$) average standards for 2.5-µm particulate matter ($PM_{2.5}$).[47] CO_2 levels were below the OSHA permissible exposure limit of 5000 ppm by volume (0.5% concentration) as an 8-h time-weighted average,[46] and NH_3 levels were below the OSHA recommended 8-h time-weighted average of 25 ppm.[8] Mean room O_2 levels were all approximately 21%, similar to reported normal atmospheric oxygen concentrations.[45]

Evaluation of microenvironmental parameters revealed few significant differences between RS. The higher mean RH over a 2-wk cage change period in RS2 cages as compared with RS1 and RS3 cages was possibly due to the low air-exchange rate of RS2, given that low ventilation rates have been reported to result in higher cage humidity levels.[29,38,48] Although NH_3 levels were not significantly different between RS, a positive relationship was found between days after cage change and NH_3 levels (that is, NH_3 levels increased over time), consistent with previous studies.[6,17,25] This effect likely was due to the accumulation of urine and urease-producing bacteria, which are known to increase intracage NH_3.[18,25,49] All room NH_3 levels were significantly lower than those of the cages, indicating effectiveness of air circulation within the racks and the room. OSHA's recommended exposure level for NH_3 is 25 ppm as a time-weighted average,[8] which was exceeded in cages of all 3 RS by day 8, whereas room NH_3 levels remained under this limit. OSHA has set 5000 ppm (0.5% concentration) as the permissible exposure limit for CO_2 as an 8-h time-weighted average,[46] and cage CO_2 levels exceeded 0.5% at all time points for all 3 systems but all room CO_2 levels remained below this limit. Cage O_2 levels showed at least a 0.5% reduction from ambient O_2 concentrations at all time points for all 3 systems. One study reported that a 0.5% reduction from ambient O_2 concentrations was coupled to alterations in mouse RBC indices indicative of chronic exposure to low-grade hypoxia.[53] Thus, regardless of the RS used, researchers should consider the potentially significant effects of chronic low-grade hypoxia on experimental results.[31,53] Unfortunately and in contrast to human gas (NH_3, CO_2, O_2) exposure standards, the lack of such standards specifically for rodents limit interpretation of our results. However, our mice were healthy throughout the study, thus indicating that their microenvironment may be acceptable. Indeed, aberrant conditions based on governing or regulatory body recommendations for human exposures may not necessarily be appropriate for mice. Wild mice normally inhabit underground burrows, where they likely are exposed to higher levels of waste gas pollutants, such as NH_3 and CO_2.[6,7,13] To date, a literature search revealed no publications investigating common waste gas exposure levels of wild mice. Such studies could provide insight into the potential waste gas exposure limits that mice might be adapted to tolerate. Although natural exposure levels have not been determined, laboratory investigations suggest potential effects of IVC housing conditions on mouse behavior[5,24,28,30,53] and waste gas pollutants on nasal pathology.[7] As such, an important study limitation is the lack of behavioral and histopathologic assessment of mice in the HD RS of this study, although future investigation could provide additional insight on potential behavioral and histopathologic correlates of the microenvironmental changes noted in this study.

Overall, few significant differences were found between RS. A few stain- or stock-dependent differences in reproductive performance were detected, but those differences were not maintained when data from the entire colony were compared across RS. Therefore, any of the 3 HD RS could provide appropriate housing conditions for mice. Further studies might include comparison of HD RS with traditional (i.e., nonHD) RS and evaluations of noise and vibration, which were beyond the scope of this investigation. In addition, research should be

performed to establish optimal microenvironmental conditions for mice, especially to guide the creation and implementation of standards, because current standards are derived based on human exposures and macroenvironmental conditions. Given that institutions conduct valuable animal research and invest in equipment, novel RS designs should be evaluated as in the current study to ensure animal and personnel welfare and research rigor and reproducibility.

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