Short Communication

Efficacy of Filter Top Cages to Reduce Cross-Contamination Between Mice in Experimental Facilities

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Abstract | Contamination is difficult to avoid during long experimental procedures when animals spend time outside their enclosures, in contact with users and different equipment. To evaluate the efficacy of filter-top cages to minimize cross contamination when experimental mice of different microbiological status share the same room, we studied a complete panel of microorganisms in mice housed in different systems. A batch of external SPF, C57BL6/N mice was split in 2 groups and housed in similar cages with or without the filter-top. These external mice shared the room with conventional mice involved in other experimental protocols between 8 and 13 weeks. Samples from different organs were analyzed by PCR. All the bacteria present in the conventional mice were also found in mice originally SPF housed in the open cages. Also, 2 out of the 6 viruses present in the facilities were transmitted to the external mice in open cages, whereas no parasites were detected. Interestingly, external mice housed in the filter-top system only recovered 3 bacteria from all the microorganism present in the facilities. Filter-top cages for long periods may be a refinement tool when there is no alternative to share the room with mice of different microbiological status.

Keywords | Experimental mice, Health monitoring, Microisolator, Refinement, Health status, Neuroscience

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INTRODUCTION

From the first wooden and glass cages used to house laboratory mice to the modern individual ventilated cages (IVC), the design of mice microenvironment has evolved to promote animals' physical comfort as well as to enable effective sanitary maintenance (U.S. Department of Health, Education, and Welfare, PHS, 1963). A milestone in laboratory animal science took place in 1958, when transmission of epidemic diarrhea of infant mice was controlled by housing mice in cages fashioned of galvanized wire mesh covered with fiberglass insulation serving as a passive air filter (Kraft, 1958). This principle was improved in several ways until the first transparent plastic shoebox with filter paper inserts on the top, commercially available in 1982 (Hessler, 1999). Although this type of cage allows to keep filtered air inside the cage, some reports warned against increases of ammonia levels in cage environments (Broderson *et al.*, 1976; Gamble and Clough, 1976; Schoeb *et al.*, 1982). Therefore, these microisolator cages were then

placed on mass air displacement racks, improving the microenvironment parameters. The ultimate step in housing conditions was provided by the positive, individually ventilated microisolation caging system, with air actively filtered before going into the cages. Most modern breeding facilities employs IVC to house mice colonies, but this is not always warranted in experimental facilities, and even less in LMIC institutions.

Several studies evaluated the efficacy of these modern types of housing systems to prevent contamination, with results varying depending on the type of agent. Proliferation of *Mycoplasma pulmonis* in the lungs of mice experimentally infected was not significantly different between filter-top cages and IVC (Booth *et al*., 2014). Another study showed no alteration in morbidity caused by *Corynebacterium bovis* in immunodeficient mice housed either in filter-top or wire-top cages (Dole *et al*., 2013). On the contrary, a partial effect of filter-top cages in preventing transmission of Mouse Hepatitis Virus (MHV) among mice was demonstrated (Macy *et al*., 2002). Likewise, transmission of MHV after 8 weeks of exposure to infected animals housed in open cages, was verified in mice housed in open cages, whereas only mice in 2 out of 10 filter-top cages seroconverted, and no mouse housed in IVC was positive (Lipman *et al*., 1993). Importantly, beside the housing system, husbandry techniques may substantially differ among facilities, influencing the final results. Remarkably, no report has been published up to now, comparing the microbiological status of animals housed in different enclosures.

The health status of experimental animals is critical because microorganisms present in laboratory animals, even at a subclinical level, can widely affect experimental results (Mähler *et al*., 2014). Housing systems have evolved to improve animal welfare, but also to provide better barriers for avoiding microbiological contamination. In this sense, IVC represent the most sophisticated technology for preventing the transmission of airborne pathogens. However, several reports point to specific alterations in the neurobehavioural phenotype of laboratory mice depending on the housing system. C57BL/6JArc mice raised in IVC systems showed more anxious-like behaviors and were socially more active than mice housed in the classic filter-top cages (Logge *et al*., 2013). The same laboratory demonstrated that schizophrenia-relevant endophenotypes previously validated in transgenic mice housed in filter-top cages were altered when mice were allocated in IVC housing (Logge *et al*., 2014). Another study in a transgenic mouse model for amyotrophic lateral sclerosis found that although central behavioural deficits were evident in both types of housing, responses were (moderately) impacted by IVC housing compared with filter-top cages (Guerra *et al*., 2021). More recently, disadvantages on the behavior and metabolism of BALB/c mice have been shown to be induced by IVC versus open cages, from an animal welfare perspective (Celik et al., 2025). Therefore, although IVC housing improved the control of microorganism transmission, alterations on behavioural traits have been also described.

The use of mice of known microbiological status is key to ensuring reproducibility of experimental results. Nevertheless, it is not always possible to keep the microbiological status of experimental animals along the procedures, even more when procedures last weeks or months. Contamination of experimental animals can be minimized by employing biosecurity measures, following standardized operative procedures, and maintaining equipment sanitation, and personal hygiene routines. Our laboratory is devoted to the field of neuroscience, conducting behavioural, neurochemical, and histological assays. Given that our experimental facility receives mice from different vendors or laboratories, and that animals are housed in open cages, it is difficult to keep mice free of microorganisms. We define, therefore, a refinement strategy to minimize cross contamination, by employing filter-top cages. SPF-certified mice were brought to our institutional facility and a health monitoring was conducted 2-3 months later, proving that the strategy was effective in limiting contamination, specifically for viruses and parasites.

MATERIALS AND METHODS

Animals

Twenty-seven mice were used for the whole study. All of the animals were involved in unrelated, non-invasive studies, mainly as breeders. Fifteen male and female C57BL6/N elite mice were purchased at the *Instituto de Medicina Experimental*, (IMEX) *Academia Nacional de Medicina*, Buenos Aires, Argentina. The remaining 12 mice, were male and female C57BL6/N mice born at the facilities of the *Instituto de Biología Celular y Neurociencia (IBCN), Facultad de Medicina, Universidad de Buenos Aires*. Experiments were conducted according to local regulations and were approved by the Institutional Ethical Committee (UBA Resol. 1848/2023 FMED). Seven weeks-old mice, bred in barrier-conditions to maintain an SPF status (as certified by Charles River), were transported in environmental controlled conditions from the IMEX to our institute animal facilities. When arrived, a group of mice (n=6; 3 mice per cage; Group 1) were immediately housed in 1284L Eurostandard Type II Long (365 x 207 x 140 mm) Tecniplast microisolator cages with filter tops covered with polyester filter sheets (atmospheric dust efficiency of 92% on 8-10 um particles) whereas another group (n= 9; Group 2) were housed in the same cage model but without

the filter-top (2-3 mice per cage). Mice stayed isolated for a week in the quarantine room before moving to the stock room, where they shared classical (non-ventilated) racks with mice involved in other experimental protocols. Animals were maintained under controlled conditions, i.e., 22 ± 2 ˚C room temperature, 60% relative humidity, 12–12 h light–dark cycle (lights on at 7.00 am). Mice received *ad libitum* pelleted food for rodents (*Cooperación*) and tap water. White pine shavings were used as bedding and 8-10 g of tissue paper was added as nesting material in all the cages. Some of these mice were used as breeders, and their litter employ in other projects. The 12 mice born at the IBCN facilities were used in different experiments or as breeders and were housed in polycarbonate open cages (300 x 200 x 140 mm; 2-3 mice per cage; Group 3).

External animals were provided with a health monitoring from Charles River corresponding to the molecular diagnostic by polymerase chain reaction (PCR) assays covering several pathogen-specific genomic sequences, all of which were negative. The IBCN facilities regularly receive animals from different vendors or research facilities, and no routine health monitoring is done. However, arriving animals must bring their corresponding health monitoring report and they remain in the quarantine room for one week under observation of technical staff. These facilities do not breed mice, except from certain transgenic lines.

Routine procedures

Mice housed in the microisolators (with or without filter-tops) were exclusively manipulated by the researchers in charge, wearing lab coats, gloves, face masks, and head covers as personal protective elements. The facility staff did not handle these animals. Cages were changed twice weekly, manipulating filter-top cages first and then, the open ones. Additionally, cages were opened on a common bench, once a day every day when female breeders were pregnant or gave birth. Microisolators were not changed inside a changing station, but on a bench. Cage changing and manipulation of mice were conducted in the same room where animals were housed. Bedding and nesting material were autoclaved at 134 ºC for 10 minutes and stored in the corridor at the entrance of the washing area. Cages and bottles were manually washed with detergent and disinfected with bleach or ammonium (monthly alternance). Filter sheets were washed with detergent and disinfected with bleach only once, at the beginning of the experiment. Food and tap water did not receive any decontainment treatment. Food bags were kept in a stock room and although a small food container was available in each animal room, feeders in microisolators (Groups 1 and 2) were weekly refilled directly with pellets taken from the food bags.

Experimental procedures

Experimental groups were defined as follows: Group 1: external SPF mice housed in filter-top cages; Group 2: external SPF mice housed in microisolators without filter-tops; Group 3: conventional mice born at our facilities housed in open cages (Table 1). External SPF mice stayed in our facility between 8 and 13 weeks, whereas mice born at our own facility were between 8 week- and 1 year-old (Table 1) at the moment we used them. At the end of the experiment, animals were killed by cervical dislocation and samples were immediately collected from the lungs, salivary glands, liver, spleen, and cecum. Fresh stool was also recovered from the rectum. Samples were kept at -60 ˚C until shipping for microbiological analysis. Samples from mice of the same experimental group obtained at different time points were pooled for the microbiological analysis in 3 different tubes: (1) feces, (2) cecum, and (3) the 4 other organs as requested by the *Laboratorio de Animales de Experimentación* (LAE), *Facultad de Ciencias Veterinarias*, *Universidad Nacional de La Plata*, the national reference center where the health monitoring was conducted. Following international recommendations (Albers *et al.*, 2023; Mähler *et al.*, 2014) and local prevalence of microorganisms, several bacteria, viruses, and parasites, as shown in Tables 2 to 4, were evaluated by PCR testing, a technique with high specificity and sensitivity. The microbial status of external mice was not confirmed upon receipt.

Table 1: Description of individual and housing conditions of experimental groups.

Experimental group #	Origin	Time spent at IBCN facility	Cage type	Sex (M:F)	Age at EEP[3]
1	IMEX[1]	8 weeks	Microisolator with filter-top	3:0	15 weeks
1	IMEX	10 weeks	Microisolator with filter-top	0:3	17 weeks
2	IMEX	8 weeks	Microisolator open	3:2	15 weeks
2	IMEX	13 weeks	Microisolator open	0:4	20 weeks
3	IBCN[2]	8-12 weeks	Open	1:5	8-12 weeks
3	IBCN	1 year	Open	3:3	1 year

[1]IMEX, external barrier facility at which mice were purchased. [2]IBCN, animal facility of our institute. [3]EEP; Experimental endpoint.

Table 2: PCR assessment of bacterial presence in samples from the 3 experimental groups coming from different facilities and bred under distinct housing conditions.

Bacteria	PCR		
	SPF - Filtertop	SPF – Open cage	Conventional- Open cage
Bordetella bronchiseptica	+	+	+
Bordetella pseudohinzzi	-	-	-
Filobacterium rodentium (CAR bacillus)	+	+	+
Citrobacter rodentium	-	-	-
Campilobacter spp.	-	-	-
Clostridium piliforme (Tyzzer)	-	-	-
Francisella tularensis	-	-	-
Corynebacterium bovis	+	+	+
Corynebacterium kutscheri	-	-	+
Helicobacter spp (H. hepaticus; H. bilis; H. rappini)	-	+	+
Klebsiella oxytoca	-	-	-
Klebsiella pneumoniae	-	-	-
Rodentibacter spp. *(R. heylii; R. pneumotropicus; R. ratti) (ex-Pasteurella pneumotropica)*	-	-	-
Mycoplasma pulmonis	-	-	-
Listeria monocytogenes	-	-	-
Pneumocystis spp. (*P. carinii; P. mutina*)	-	+	+
Salmonella spp.	-	-	-
Shigella spp.	-	-	-
Streptococcus pneumoniae	-	-	-
Streptobacillus moniliformis	-	-	-

Qualitative analysis

The purpose of this work was to evaluate the presence or absence of specific microorganisms without statistical comparisons among groups. For the qualitative analysis, samples from different animals of the same group were pooled at the request of the laboratory conducting the screening (LAE). Depending on the presence or not of each microorganism, the experimental group was considered positive or negative for this agent.

RESULTS

The colonies of origin of the external mice were certified to be negative for all the microorganisms studied. Health monitoring results for the conventional mice born at the IBCN (Group 3) were positive for several bacteria, virus, and parasites (Tables 2-4). All of the bacteria present in this control group, except for the *C. kutscheri*, were also found in the group of mice originally SPF that were housed in the open cages (microisolators without filter-top) for 8 to 13 weeks (Group 2; Table 2). Concerning the viruses, only 2 out of the 6 present in the facilities were transmitted to the mice in open cages (Table 3), whereas no parasites were detected after 2 or 3 months of sharing the room (Table 4). Interestingly, mice housed in the filter top system (Group 1) only recovered 3 bacteria from all the microorganism present in the facilities (Table 2).

Mice in Groups 1 and 2 were also used as breeders. Although the analysis of reproductive parameters did not reach relevance to be statistically analyzed, performance of mice in either group (i.e. fertility, mice per litter, and weaned mice per mother) was similar independently of the housing system.

DISCUSSION

Whereas mice in breeding facilities are usually reared free of specific pathogen, the microbiological status of animals in experimental conditions is more difficult to maintain as they are in contact with users, different equipment or apparatus, and may spend time outside their cages. In this study we analyzed the microbiological profile of SPF mice allocated in filter-top cages, sharing the room with mice contaminated with several bacteria, viruses, and parasites. Although no extra procedures were put into practice, the filter-top cages appear to be key to control transmission of viruses, parasites, and most of the bacteria after, at least, 8 weeks of sharing the room with conventional mice. Some other procedures like facility staff not handling mice in

Table 3: PCR assessment of viral presence in samples from the 3 experimental groups coming from different facilities and bred under distinct housing conditions.

Virus	PCR		
	SPF -Filtertop	SPF – Open cage	Conventional-Open cage
Mouse adenovirus (*MAd-1*; *MAd-2*)	-	-	-
Pneumonia virus of mice (*PVM*)	-	-	+
Mouse astrovirus (*AstV tipo 1- 2*)	-	+	+
Sendai virus (*SeV*)	-	-	-
Murine norovirus (*MNV 1-4*)	-	+	+
Minute virus of mice (*MVM*)	-	-	-
Lactate dehydrogenase elevating virus (*LDH*)	-	-	-
Lymphocytic choriomeningitis virus (*LCMV*)	-	-	-
Mouse parvovirus (*MPV*)	-	-	+
Mouse rotavirus (*RMV*; *EDIR*)	-	-	-
Hantavirus (*Hanta*)	-	-	-
Citomegalovirus (*CMV*)	-	-	-
Mouse polyoma virus	-	-	-
Theiler's murine encephalomyelitis (*GDVII*; *FA*)	-	-	+
Reovirus (*Reo 1-4*)	-	-	+
Mouse hepatitis virus (*MHV1-3*; *JHM*; *A59*)	-	-	-
Mousepox (*Ectromelia*) *virus*	-	-	-

Table 4: PCR assessment of presence of parasites in samples from the 3 experimental groups coming from different facilities and bred under distinct housing conditions.

Parasites	PCR		
	SPF - Filtertop	SPF – Open cage	Conventional – Open cage
Aspiculuris tetraptera	-	-	-
Giardia muris	-	-	+
Criptosporidium spp.	-	-	+
Hymenolepis spp. *(H. diminuta, Rodentolepis nana)*	-	-	-
Encephalitozoon cuniculi	-	-	-
Spironucleus muris	-	-	-
Toxoplasma gondii	-	-	-
Syphacia spp. *(S. obvelata, S. muris)*	-	-	+

microisolators (Group 1 and 2 mice were exclusively manipulated by the researchers in charge) or refilling of feeders with pellets directly obtained from food bags, may be important for the interpretation of our results, as parasites present in conventional animals (Group 3), as well as 4 out the 6 viruses (*PVM*, *MPV*,*GDVII*, and *Reo1-4*) and 1 bacteria (*C. kutscheri*) were not transmitted from conventional mice to originally SPF mice housed in microisolators without filter-tops. As latent or unapparent infections, can have a considerable impact on experimental outcomes (Mähler *et al*., 2014), this housing system can help to maintain an optimal microbiological status while conducting research in facilities in which animals are positive for a list of microorganisms. By minimizing cross contamination between cages, we were able to improve the welfare of the mice employed and thus, refine our experimental procedures.

The use of IVC combined with change stations may be the optimal way to avoid cross contamination as well as allergen exposure (Feistenauer *et al*., 2014), but this equipment requires specific design of the facilities and have a high startup cost. On the other hand, filter top cages handled in change stations has been demonstrated to efficiently prevent contamination of most viruses and parasites (Wiese *et al*., 2007). Filter top cages have been also proved effective in reducing airborne allergen levels (Gordon *et al*., 1992; Hollander *et al*., 1998), but it has been indicated that accumulation of the waste gases carbon dioxide (CO_2) and ammonia (NH_3) could occur (Lipman *et al*., 1992). To avoid

accumulation of these gases, we increased the frequency of bedding change, i.e., twice a week, and adopted low animal density. In addition, filter-top vendor claims that due to the large filtration area of the filter top systems, the filter tops maintain an efficient gaseous interchange with the external environment, resulting in lower levels of intra-cage NH_3 and CO_2. Indeed, we checked animals daily and no signs of distress were detected nor we do smell ammonium. Polyester sheets, used as filter tops in our cage model, are primarily used as pre-filtration in the HVAC equipment as they remove larger particles. As advantages, these filters are cheaper, moisture-resistant, and washable. High-efficiency particulate air (HEPA) and ultra-low penetration air (ULPA) filters are cutting-edge filtration systems, removing, respectively, a minimum of 99.97% of 0.3 µm, and 99.999% of 0.12 µm particles from the air. Typically, HEPA filters medium is a single sheet of borosilicate fibers treated with a wet-strength, water-repellant binder, but other medias, like teflon (polytetrafluoroethylene), have been developed to improve the filtration capacity (Meechan and Potts, 2020). Although HEPA-filtered airflow to IVC prevents the entry of microorganisms to the microenvironment, these filters as well as ULPA filters are denser, thus creating airflow resistance (Hogan *et al*., 2018). More modern fiber materials like polyphenylene sulfide, polyamide fiber, ceramic, and metal, that have better physical characteristics (Diao and Yang, 2021) have not still been essayed for their use in rodent cages. In addition, airborne respiratory pathogens, like the emerging *SARS-CoV-2*, require improvements in materials and technologies to prevent the spread of these contaminants. For example, filter fibers treated with a novel biocide coat, chlorhexidine digluconate, has been proved to have antimicrobial and antiviral activity (Watson *et al*, 2022). Although filter tops have been developed to control the quality of the air in the microenvironment, no studies have been published to date that show the effectiveness of limiting transmission of pathogens in long-term trials with mice. All in all, our results show that using filter top cages for long periods of time may be a refinement tool when experimental mice of different microbiological status need to share the room. As infections, apparent or unapparent, may confound scientific results and increase the number of animals used, the screening of affordable refinement techniques to improve animal welfare adds a plus to animal experimentation.

Limitations

Although the 10 minutes' transport of animals between the supplier and our institution were done in cardboard boxes with ventilation through filters, we cannot exclude that mice could have got contaminated during the transport, as no extra pathogen screening was conducted upon arrival of mice.

Filter sheets in the filter tops are washable, but we did not change or wash them during the period that the experiment lasted. Therefore, a decrease in efficiency may have occurred along the weeks. Changes and wash of the filters every 2 weeks should be more appropriated in order to maintain their efficacy as passive filters.

CONCLUSION

The results described in this study demonstrate that, in absence of more modern housing systems, filter-top cages used for long periods become a refinement tool when mice of different microbiological status have to share the same room.

NOVELTY STATEMENT

IVC may provide advantages concerning the quality of the air inside the cages, but no study has analyzed up to now, the microbiological status of mice housed in different enclosure systems. Our study shows that filter-top cages became a refinement tool when there is no alternative to share the room with animals of different microbiological status for several weeks.

AUTHOR'S CONTRIBUTION

DRM and KMS conducted the work with the animals and recovered the samples. JA, MC, and JML performed the microbiological analysis and interpretation of results. DRM and SLD conceived the experiments and wrote the manuscript. All the authors revised the manuscript. SLD provided the funding and coordinated the activities.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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