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Efficacy of mini-containment units in isolating mice from micro-organisms.

by *J.M. Morrell.*
European Molecular Biology Laboratory, Meyerhofstrasse 1,
Postfach 10.2209, D-69012 Heidelberg, Germany.

Introduction

Transgenic mice are frequently exchanged between scientists (GV-SOLAS, 1996), necessitating their transport between animal houses. They will quickly share their microbiological flora with the inhabitants of the receiving animal unit, unless some measures are taken to isolate the two groups of mice. Mini-containment equipment, for example ventilated cabinets and individually ventilated cages, which serve to separate the animals from potential pathogens in the environment, could assist the quarantining of small groups of incoming mice in the same facility and help to isolate the mice from each others' microbiological flora on a long-term basis (*Clough*, 1995).

The mice are housed in filter-top cages which are supplied with filtered in-coming air. They remain protected from organisms in the environment while the cages are in position either on the rack or in the ventilated cabinet. Theoretically there is the risk of transfer of infection when the cages are opened to work with the mice or for husbandry activities. Therefore such activities should be carried out in a sterile laminar flow hood or change station and must take place under carefully controlled conditions to reduce the possibility of infection being introduced. However, the real risk of cross-contamination occurring between 2 groups of mice with different microbiological flora can only be assessed in practice and is presumably dependent on the pathogen(s) concerned.

This report describes the outcome of housing two groups of mice with different bacterial flora in mini-containment facilities in the same room over a period of 7 months.

Materials and Methods

Animals and Husbandry

In July, transgenic mice were transported from the United States to Germany in filtered boxes. They were housed in individually ventilated cages (IVC rack, Techniplast; obtained from Scanbur, Køge, Denmark). Irradiated aspen bedding and nesting material (Tapvei, Kortteinen, Finland) were provided in the filter-top cages on the rack. Acidified water, automatically controlled and mixed to pH 2.9, and irradiated rodent breeding diet (RM3; Special Diet Services, Boxmeer, The Netherlands) were available ad libitum. The environmental conditions in the room (21° ± 2° C, relative humidity 55 ± 10%) and the light : dark cycle (12 hour light, 12 hour dark) were monitored continuously by computer. All servicing of cages and handling of mice took place in a laminar flow hood in which an ultraviolet light was switched on for at least 15 minutes before use. The hood was thoroughly cleaned with Virkon (Schah-Zeidi; Altenstadt, Germany) after use. Personnel always wore gloves when handling the mice, spraying the gloves with Virkon after touching the cage or anything outside the laminar flow hood. Entrance to the room was restricted to the animal house personnel responsible for the mice and the scientific staff working with the mice.

Six months later, female mice of a different transgenic line, of known health status, were removed from an isolator and housed in filter-top cages (B type cage; Scanbur, Køge, Denmark) within a ventilated cabinet (Scantainer; Scanbur, Køge,

Denmark). Male mice carrying yet another transgene were transported from another institute in filtered boxes and were housed in the same ventilated cabinet. After an acclimatisation period of one week, breeding pairs were set up. The husbandry conditions were the same as for the mice in the IVC racks. Since only one laminar flow hood or work station was available, the standard operating procedure for this room was altered to accommodate an additional de-contamination step: if it was necessary to work with mice from the IVC racks and from the ventilated cabinet on the same day, the laminar flow hood was cleaned thoroughly with Virkon and the UV light was switched on for at least 15 minutes before starting work with the second set of mice.

Health screening.
Mice from the IVC racks were screened 3, 6, 8, 9 12 and 14 months after arrival (*Prof. Needham*; The Microbiology Laboratories, London, UK) according to FELASA guidelines (*Kraft et al.*, 1994). On each occasion 5-12 mice were selected at random from the IVC racks. In addition, after 6 months a full health screen was carried out on 6 adult wild-type littermates from the ventilated cabinet with a further screen being performed on 6 mice one month later. All necropsies were performed, and samples taken and processed according to standard procedures (*Needham*, 1979).

Results
Mice in IVC racks
In all mice from the IVC racks (total 57 mice), virus antibodies were absent (Table 1), no antibodies to *Mycoplasma pulmonis* or *Clostridium piliforme* were found and microscopic agglutination tests for *Leptospira* serogroups *ballum, canicola, hebdomadis* and *icterohaemorrhagiae* were negative. Furthermore, no ectoparasites were detected. However, in the screen made after the mice had been housed for 6 months in the IVC racks, Enteromonas species were seen for the first time in the caecal contents of 5 out of 12 mice. In subsequent screens, this protozoan was seen in 2/12, 6/10, 3/8 and 5/8 mice.

There was a change in the bacterial species cultured from the mice in the screens performed between 6 and 14 months after arrival (Table 2), with seven new ones being detected over this time period. The bacterium which was potentially a problem with regard to pathogenicity, *Pasteurella pneumotropica*, was first identified in 1 out of 12 animals screened 8 months after arrival. At the following screen (9 months after arrival), *P. pneumotropica* was cultured from 6 out of 10 mice but a subsequent screen (12 months after arrival) showed only 1 out of 8 mice to be infected. By 14 months after arrival, 2 out of 8 mice were found with *P. pneumotropica*. No clinical signs of respiratory disease were observed in the mice housed in the IVC racks and there was no evidence of lung pathology at necropsy.
With the exception of one positive animal, whose background is unfortunately unknown and has therefore been removed from the calculation, the genotype of all the mice found with *P. pneumotropica* was based on a Balb/c background (9 out of 38 animals) while none of 18 mice with a BL6 background was found to be contaminated with this bacterial species, although *P. haemolytica* was cultured from 2 animals. However, the difference is not statistically significant (Chi squared test).

Table 1: Results of the serological examination of mice housed in IVC racks for a 14 month period.

Pathogen and test method	3 months	6 months	8 months	9 months	12 months	14 months
MVM (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
PVM (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
MHV (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Reo 3 (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
TMEV (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Sendai (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Adenovirus (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Ectromelia (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
LCM (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Polyoma (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Rota virus (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Cytomegalovirus (ELISA)	0/7	0/12	0/12	0/10	0/8	0/8
Hantavirus (IFA)	0/7	0/12	0/12	0/10	0/8	0/8
K virus (IFA)	0/7	0/12	0/12	0/10	0/8	0/8
Thymic virus (IFA)	0/7	0/12	0/12	0/10	0/8	0/8

Note: ELISA tests for antibodies to *Clostridium piliforme* and *Mycoplasma pulmonis* were also negative.

Table 2: Bacteria cultured from hybrid mice housed in IVC racks over a 14 month period.

Organism	3 months	6 months	8 months	9 months	12 months	14 months
Proteus mirabilis	4/7	3/1	2/12	3/10	0/8	178
E. coli	4/7	11/12	8/12	9/10	5/8	6/8
Staphylococcus spp.	4/7	12/12	5/12	3/10	2/8	(78
a haemolytic streptococcus	7/7	12/12	12/12	10/10	8/8	7/8
Enterobacter sp.	1/7	0/12	0/12	0/10	0/8	0/8
Enterococcus		3/12	1/12	0/10	1/8	1/8
Vibrio sp.			1/12	0/10	1/8	0/8
Pasteurella pneumotropica			1/12	6/10	1/8	2/8
Gardneella sp.				1/10	0/8	0/8
Oerskovia sp.				1/10	0/8	0/8
Pasteurella haemolytica						2/8
Corynebacterium sp.						1/8

Mice in Scantainer

The results of serological screening for viral antibodies from mice before and after housing in the Scantainer showed that the mice were virus antibody free (VAF) (Table 3). Again, tests for antibodies to *Leptospira* subgroups were negative and no parasites were found in any of the mice. There was little change in the bacteria cultured from these sentinel mice over the 7 month period, as shown in Table 4, even though this period coincided with the time period over which additional bacterial species were found in the mice in the IVC racks.

Table 3: Results of the serological examination of mice before and after housing for 6 and 7 months in a ventilated cabinet.

Pathogen and test method	Before	After 6 months	After 7 months
MVM (ELISA)	0/4	0/6	0/6
PVM (ELISA)	0/4	0/6	0/6
MHV (ELISA)	0/4	0/6	0/6
Reo 3 (ELISA)	0/4	0/6	0/6
TMEV (ELISA)	0/4	0/6	0/6
Sendai (ELISA)	0/4	0/6	0/6
Adenovirus (ELISA)	0/4	0/6	0/6
Ectromelia (ELISA)	0/4	0/6	0/6
LCM (ELISA)	0/4	0/6	0/6
Polyoma (ELISA)	0/4	0/6	0/6
Rota virus (ELISA)	0/4	0/6	0/6
Cytomegalovirus (ELISA)	0/4	0/6	0/6
Hantavirus (IFA)	0/4	0/6	0/6
K virus (IFA)	0/4	0/6	0/6
Thymic virus (IFA)	0/4	0/6	0/6
Mycoplasma pulmonis (ELISA)	0/4	0/6	0/6
Clostridium piliforme (ELISA)	0/4	0/6	0/6

Table 4: Bacteria cultured from mice before and after housing in a ventilated cabinet for 6 and 7 months.

Organism	Before ð	After 6 months °	After 7 months §
Proteus mirabilis	3/4	4/6	4/6
E. coli	3/4	476	4/6
Staphylococcus spp.	1/4	5/6	6/6
a haemolytic streptococcus	1/4	5/6	476
Enterococcus	3/4	0/6	1/6
Corynebacterium sp.	1/4	1/6	0/6

Note: ð 4 adult hybrid mice; ° 6 adult hybrid mice; § 6 adult hybrid mice.

Discussion

The results of this study show that it was possible to maintain the health status of mice housed in a ventilated cabinet in the same room as mice in IVC racks which were known to be contaminated with a potential pathogen, without transmission of the organism between the two groups of mice. Although mice in the IVC racks did become contaminated with several bacterial species, including *P. pneumotropica*, and the protozoan Enteromonas, these organisms were contained and did not spread to the mice in the ventilated cabinet.

The lack of clinical signs of respiratory disease or lung pathology observed in the mice from the IVC racks supports the opinion that *P. pneumotropica* is an opportunistic pathogen rather than a primary invader (*Jacoby & Fox*, 1984). It is now thought that clinical cases, in which *P. pneumotropica* is found to be present, may result as a complication of pneumonias caused by, for example, *Mycoplasma pulmonis* or Sendai virus (*Harkness & Wagner*, 1983: *Jacoby & Fox*, 1984). The detection of this organism, and of 6 other new organisms over the 8 month period, raises the question of how the mice in the IVC racks could have become contaminated. It would be important to identify potential sources of contamination since, presumably, pathogens could enter by the same route.

The route of contamination in this case is not known. It is possible, but is considered unlikely, that the mice were already contaminated before they arrived at EMBL. A health screen of mice from the originating unit did not reveal *P. pneumotropica*: 7 mice out of approximately 350 were checked at the first health screen 3 months after arrival and 12 out of approximately 700 were screened 6 months after arrival, with no *P. pneumotropica* being detected. However, all personnel working with the mice thought they had closely followed the standard operating procedure for that room, which included the wearing of masks at all times and prompt changing of torn gloves (Appendix 1). If the standard operating procedure was not being followed, it might be expected that the mice in the Scantainer would have become contaminated too, but these mice have remained free of additional organisms until the time of writing (at least 7 months) even though they are housed in the same room and the same changing station is used for both sets of mice. Furthermore, these organisms were not isolated from any rodents housed in other rooms in the animal house over the same period. Therefore, regardless of the method of contamination, the IVC racks were effective in containing the organisms once the mice had become infected.

In conclusion, mini-containment units such as IVC racks and ventilated cabinets offer considerable potential for aiding mouse husbandry and controlling the spread of potential pathogens, by effectively isolating small groups of animals. However, it is vital to ensure that all standard operating procedures are closely followed when personnel are working with animals housed in such units.

Appendix 1. Instructions for work with mice in mini-containment units.

1. Always wear a clean green gown, latex gloves, mask (M3) and hat when working in this room.

2. Turn on the UV light in the laminar flow hood for 15 min. before using the hood.

3. Make sure Virkon in spray bottle is not more than 2 days old. A fresh solution is prepared by dissolving 10 g powder in 1 liter tap water. Note: items sprayed with Virkon are adequately disinfected after 15 min.

4. Spray equipment, outer diet bags, water container etc. with Virkon before introducing into hood.

5. Always spray gloves again with Virkon after touching items outside hood e.g. cages.

6. Replace gloves immediately if torn.

7. When work in the hood is finished, remove unwanted items, wipe around thoroughly with Virkon.

8. If working with mice in IVC rack and in Scantainer, work should be completed with mice in IVC rack FIRST, switch on UV light for 15 min., change gloves, then proceed with mice in Scantainer. When work is finished, switch on UV for 15 min.

9. Put green gown in laundry bin after use.

10. When cage changing is completed for the week, animal technician should remove base plates from the hood, clean area under base plates thoroughly including drainage hole. Turn UV light on for 15 min. while washing base plates with Virkon and rinsing with clean water. Replace base plates and switch on UV again for 15 min.

11. The floor should be mopped with Virkon every day and the walls wiped down with Virkon once a week. Note: the top of the ventilation pipe collects dust: it should be washed once a week with Virkon.

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Summary
Two groups of mice with different microbiological flora were housed in the same room, one group in individually ventilated cages (IVC) and the other in a ventilated cabinet. Routine health screening carried out at regular intervals revealed that these mini-containment units were effective in preventing exchange of micro-organisms between the two groups of mice. Although the animals in the IVC racks did become contaminated with organisms from an unknown source, there is no evidence to suggest that the IVC racks did not function properly. Therefore mini-containment units offer considerable potential for controlling the health status of animals in different groups but work with such animals must take place in accordance with standard operating procedures.

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