Reduction in the spread of rodent urinary allergens during cage changing by Laminar Air Flow cabins. Reduction in rodent allergens by LAF cabins"

by ***Thomas C. Krohn* & Axel Kornerup Hansen***
Division of Laboratory Animal Science & Welfare, Department of Veterinary Pathobiology,
Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Summary
As spread of allergens, especially during handling and cage changes, is a problem in an animal facility, initiatives are taken to reduce this spread. The aim of the present study was to evaluate the effectiveness of a LAF-cabin (Laminar Air Flow cabin), and its ability to protect the staff against inhaling allergens during cage changing of rats, and mice. The allergens were sampled from the handler's breathing zone and from different places inside the cabin. Subsequently the amount of allergens was quantified by an ELISA testing for MUS m1 and RAT n1.
The use of the LAF-cabin reduced the amount of rat and mouse allergens in the breathing zone by at least 90-95%. The study shows that by using a LAF-cabin for cage changing it is possible both to achieve an essential reduction in allergen exposure for the staff and at the same time keep the allergens inside the ventilated area of the cabin, thereby preventing allergen contamination of the surrounding facilities. The potential advantages compared with other techniques are outlined.

Key words: Allergens, cage changing, exposure, Laboratory Animal Allergy, rat, mice

Introduction
Allergy to laboratory rodents is a well-known occupational health problem, and many incidents are reported annually. An increasing number of facilities are introducing policies to prevent laboratory animal allergy. From mice and rats the primary causes are the urinary proteins Mus m1 and Rat n1, respectively, but urinary albumin may also act as an allergen *(Bush et al., 1998)*. The urine contaminates the bedding from where the allergens are further spread by the animal's activity or during cage emptying *(Hollander et al., 1997)*. Also the fur may get contaminated thereby exposing animal handlers to allergens. Therefore, cage cleaning and changing as well as handling of animals are the exposure situations during which the staff should be protected against inhaling and in other ways getting into contact with allergens, which may be achieved by personal protection equipment, e.g. a dust mask or ventilated full-face mask *(Hunskaar & Fosse, 1993)*. To prevent spread of allergens, the cage changing process must take place using a ventilated bench or another kind of ventilated equipment *(Gordon et al., 2001)*. However, protection equipment may be uncomfortable, and handling or cage changing on a bench in awkward working positions may be hazardous for the staff. So ideally, proper working positions should be combined with protection against allergens. This is possible in a Laminar Air Flow cabinet (LAF cabin). The aim of the present study was to assess the effectiveness of a LAF-cabin, and its ability to protect the staff against inhaling allergens during the process of rodent cage changing.

Correspondence: Thomas C. Krohn, Royal Veterinary and Agricultural University, Division of Laboratory Animal Science & Welfare, Depart. of Veterinary Pathobiology, Grønnegårdsvej 15, DK-1870 Frederiksberg C, Denmark.
Phone: +45 35 28 38 48. Fax: +45 35 35 35 14.
E-mail: tkr@kvl.dk

Materials and Methods

The cages used for the cage changing procedure were dirty rat and mouse cages from an animal unit of a pharmaceutical company. The changing of rat and mouse cages was done on separate occasions. These Type III cages, which were changed biweekly, were placed in a ventilated Allergy Cabinet (Scanbur-BK, Denmark) connected to a central room ventilation of approximately 60 air changes per hour. Each cabinet hold 20 Type III cages and each cage contained 1-2 rats or 5-10 mice.

In the cages a layer of asp chips (Tapvei, Finland) were used, and the temperature at the inlet air was 21±1 °C and the humidity 50±5 %.

The cage changing procedures

Two different procedures were followed during cage changing. Both were conducted inside the LAF-cabin; once with the cabin turned on and once when turned off.

The first procedure followed the routine procedures of the animal unit, which were as follows:

- Cages were changed from the cabinet top, and each column was finished before starting on a new one
- No care was taken to keep the cages at arms distance from the body
- The cages were changed on an ordinary table without perforation.
- The empty cages with the dirty bedding were stacked on the table

The alternate procedure was performed according to a set of Standard Operating Procedures (SOP) issued by the manufacturer of the LAF-cabin. Using the SOP, the following special precautions were taken:

- Cages were changed from the cabinet top, and each row from the top to the bottom was changed before moving to the next row
- Cages were handled at arms distance from the body, especially from the breathing zone
- Cages were changed on a special table with a perforated table top
- Empty cages with the dirty bedding were stacked in special trolleys designed for cage changing (Scanbur-BK, Denmark)

As the studies aim was to test the efficiency of the LAF-cabinet and not necessarily the allergen load during a normal cage changing procedure, special action was taken: During the cage changing procedure, the cage changing person never left the LAF-cabin to prevent allergens from outside the cabin to contaminate the filter in the pump. Therefore another person outside the cabin replaced the ventilated cabinets whenever fully changed. The cage changing person wore a special suit to avoid spread of allergens from her dress to the filter.

The cage changing procedure for each sampling, equal to one filter, was 30 minutes and at that time three full cabinets could be changed, equal to 60 cages.

The LAF cabin

A LAF cabin 1916 (Scanbur-BK, Denmark) 1.58 metres wide and 1.91 metres deep was used (Figure 1).

Figure 1: The LAF-cabin with special table and special trolleys for cages. Both sides were covered with transparent plastic strip curtains. The table has a perforated top. The cabinet that is going to be changed is placed in the opening in the front and the door opened into the cabin.

The cabin was ventilated with 1620 m^3 per hour per m^2 through a HEPA-filter with a downwards air flow of 0.4 – 0.5 m/s. Both sides were covered with transparent plastic strip curtains.

Air sampling

Air was drawn through a filter by the use of an air sampler (Aircheck2000, SKC Inc., US) pumping 2.0 litre per minute running 30 minutes for each sample and capturing allergens on a 1.0 µm filter (FALP 02500, Millipore, Denmark) placed in a filter cassette connected to the pump through a 1.2 metres long silicone tube.

For sampling allergens, the filter was placed in one of three places:

- Attached to the person's collar just beneath the breathing zone quantifying how much allergens the person inhale
- In front of the exhaust filter of the LAF-cabin quantifying how much allergens spread during the cage changing
- In front of the inlet filter underneath the roof of the LAF-cabin for quantifying how much allergens there is in the inlet air after re-circulation through HEPA-filter

Sample analysis

The method used for elution of the filters was adopted from an established method (Renstrom, 1997). After sampling, the filters were placed in a 1.5 ml Eppendorf tube and eluted 1.0 ml phosphate buffered saline (PBS) with 0.5% Tween 20. The filters were immersed for at least two hours to ensure that all the allergens were washed off the filters. Hereafter the eluate was isolated and 0.1 gram of heat-fractionated Bovine Serum Albumin (Sigma-Aldrich A7030) was added to each sample. Then the samples were stored at -20°C until analysis. After thawing, samples were analysed for allergens by ELISA using the Mus m1 Elisa Kit and/or the Rat n1 Elisa Kit (Indoor Biotechnologies, Manchester, UK). The protocol from the Kit-set was used, excepting visualization by OPD (DakoCytomation, S2045, Denmark) and reading at 492 nm with 630 nm as reference. All samples were tested twice at the ELISA as undiluted, 1/2 diluted and 1/5 diluted. The detection limit for the samples in the present study, recalculated from the results from the ELISA and the air sampling, is 0.2 ng/m3 for Mus m1 and 2.5 ng/m3 for Rat n1.

Results

From the ELISA readings and the known air flow at the air sampling pump, the results can be recalculated and the amount of allergens in the air can be given.

The use of the LAF-cabin reduced the amount of rat and mouse allergens in the breathing zone by at least 90-95% (Fig. 2 and 3). Without applying the manufacturer's SOP a reduction in allergens at the breathing zone of approximately 90-95% was achieved, while using the SOP no allergens were detected at the breathing zone during change of rat cages. If the LAF-cabin is not turned on, the allergens spread through the entire cabin, and the allergen levels at the breathing zone is high.

Samples from underneath the roof of the LAF-cabin in front of the HEPA-filter showed that the air re-circulated into the cabin did not contain any allergens.

Discussion

By using a LAF-cabin a major reduction in the amount of allergens spread during cage changing can be achieved, equivalent to the reduction achieved by changing inside a ventilated cabinet or LAF-bench *(Gordon et al., 1997)*. However, the LAF-cabin has the advantage that the working position is normal, as the LAF-cabin is an oversize LAF-bench of walk-in size. If a person need to change many cages each day, it is important, that the working position and ergonomic is good, otherwise it may cause health problems for the person. In the LAF-cabin the person is able to work freely and the load on the body is minimised compared to working in a LAF-bench. Furthermore, the LAF-cabin enables containment of all parts of the procedure inside the LAF-cabin thereby preventing

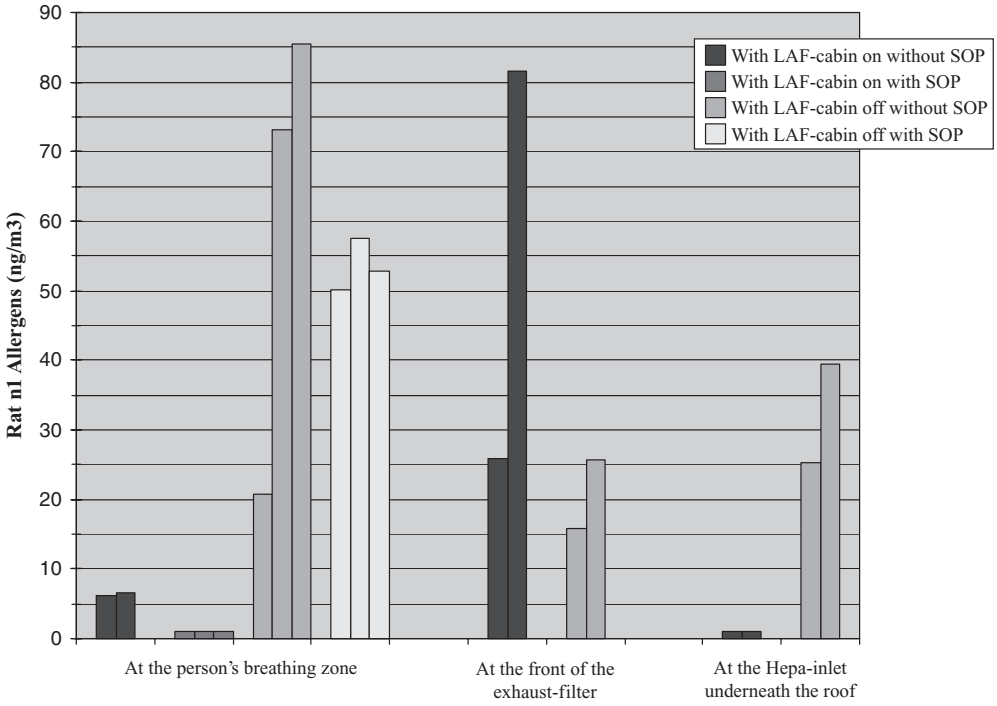

Figure 2: Results of monitoring Rat n1 allergens during cage changing. Allergens were sampled and analysed from the cage changer's breathing zone, at front of the exhaust-filter and at the air inlet underneath the roof. The results for the LAF-cabin on with SOP and one of the values with LAF-cabin on without SOP at persons breathing zone and the results for LAF-cabin on without SOP at the HEPA-filter inlet is beneath detection limit (2.5 ng/m3) and are only marked at the figure for visualising. Each column represents 30 minutes of cage changing and approx. 60 cages were changed within that time.

spread of allergens outside the cage changing area. The alternative, a ventilated cabinet , requires the dirty cages to be stacked outside and thereby it does not prevent spread of allergens to suroundings *(Thulin et al., 2002)*. Even without using the producer's SOP, the LAF-cabin seemed to be efficient in removing allergens from the process. A reduction of 90-95% in allergens when using the LAF-cabin, is a protection equivalent to using a P2 facemask *(Renstrom et al., 2002).* Using a short period for training the staff for a specific SOP may even lead to an allergen-free cage changing procedure making personal protection equipment unnecessary, as would normally be recommended *(Hunskaar and Fosse, 1993)*. The spread of allergens from handling and experimental procedures such as injections, feeding etc., during which the spread of allergens are high *(Gordon et al., 2001)*, may also be reduced by the use of a LAF-cabin. However, using a LAF-cabin is no guarantee against development of laboratory animal allergy as there is no lower threshold for a safe amount of allergen exposure *(Gordon et al., 1997)*, and the mode of development of laboratory animal allergy is unclear *(Renstrom et al., 2001)*. It is suggested that a 10-fold reduction in the amount of allergens, which is the reduction achieved in the LAF-cabin, may be considered meaningful *(Reeb-Whitaker et al., 2001)*. Also, the LAF-cabin seems to be efficient in preventing spread of allergens to other areas thereby prevent-

ing laboratory animal allergy in staff not routinely entering the animal facilities.

In conclusion, the study shows that by using a LAF-cabin for cage changing it seems possible both to achieve an essential reduction in allergen exposure for the cage changing staff and at the same time keep the allergens inside the ventilated area of the cabin, preventing allergen contamination of the surrounding facilities. The results must be confirmed by a larger study as only a few samples were used in the present study and in a larger study the exact reduction in allergens could be calculated.

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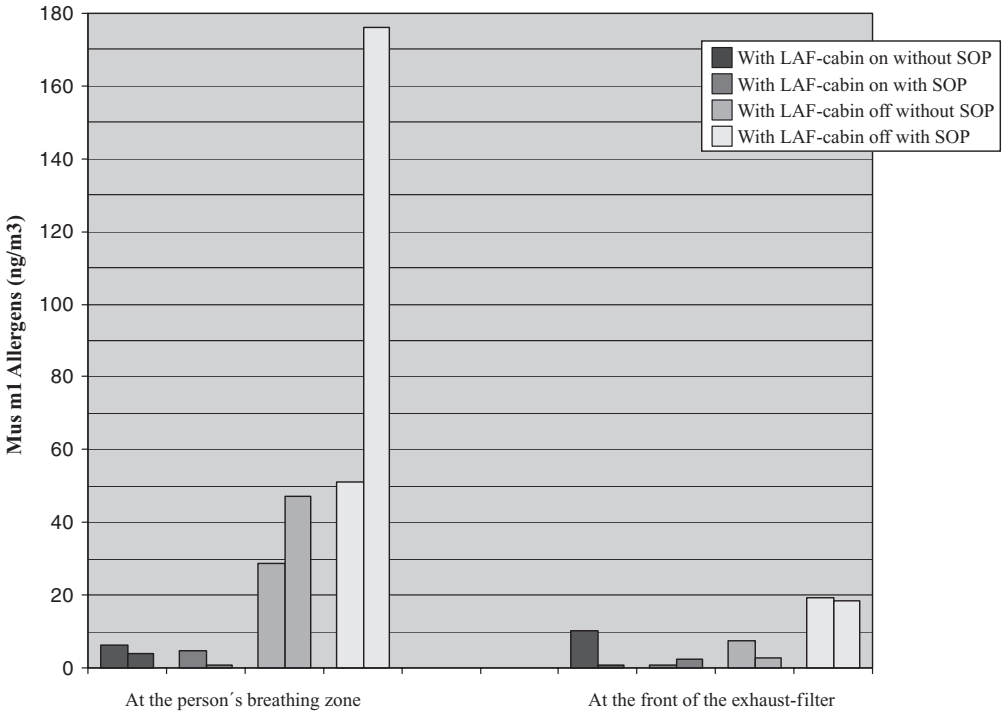

Figure 3: Results of monitoring mus m1 allergens during cage changing. Allergens were sampled and analysed from the cage changer's breathing zone, at front of the exhaust-filter and at the air inlet underneath the roof. One of the results with the LAF-cabin on with SOP in each situation and one result with the LAF-cabin on without SOP at front of the exhaust-filter are underneath detection limit (0.2 ng/m3) and are only marked at the figure for visualising. Each column represents 30 minutes of cage changing and approx. 60 cages were changed within that time.

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