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Prevention of airborne contamination and cross-contamination in germ-free mice by laminar flow

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SUMMARY

The efficacy of horizontal and vertical laminar flow units (equipped with high-efficiency air filters) in the prevention of cross-contamination between cages and of contamination from outside has been demonstrated. With germ-free mice and using germ-free standard techniques for sterilization and for the transfer of germ-free mice into the cabinets via a standard entry lock, it was found that during an observation period of 2 weeks the animals remain 'negative'. Other experiments were performed with equally good results in cabinets equipped with a hinged flap, closing 95% of the open front side. When the flap was closed the air flow could be reduced accordingly, thus reducing the noise level and the risk of dehydration.

Experiments made with germ-free mice in a 'down-flow unit' were also invariably good.

In another type of experiment, cages with conventional mice were placed in the cabinets between cages with germ-free animals at varying distances. If all animals were maintained on wire mesh (to minimize the aerosol production of dust) and if the 'conventional' cages were at a distance of 10 cm. from 'germ-free cages' the latter remained bacteria-free during test periods of one week.

The use of 'laminar flow isolators' for the isolation of human patients is mentioned.

INTRODUCTION

Infection frequently complicates experiments with animals in which immune response is decreased by irradiation, chemotherapy or the administration of anti-lymphocyte serum. In most case these infections originate from the digestive tract. By oral administration of antibiotics the digestive tract of conventional animals can be decontaminated, but in a few days these animals become highly susceptible to antibiotic-resistant strains of many bacterial species. Oral contamination with small numbers (< 100 cells) of, for example, *Escherichia coli* invariably results in a rapid and permanent 'take'.

Decontaminated animals to be used for radiation studies must therefore be isolated to prevent contamination and colonization. The standard germ-free isolator is unsuitable for this purpose because antibiotic-resistant organisms, occasionally present in one of the animals, rapidly contaminate the whole group inside the isolator (van der Waaij & Sturm, 1968). This dissemination of a con-

taminant might be prevented by distributing the animals over a number of cages in a cabinet with a 'laminar flow' system (Whitfield, 1962). In such a system, prefiltered air is blown through a 'high efficiency' (HEPA) filter and enters the working area of the cabinet through numerous small holes in a panel in the rear wall. In properly constructed benches the resulting air stream is non-turbulent.

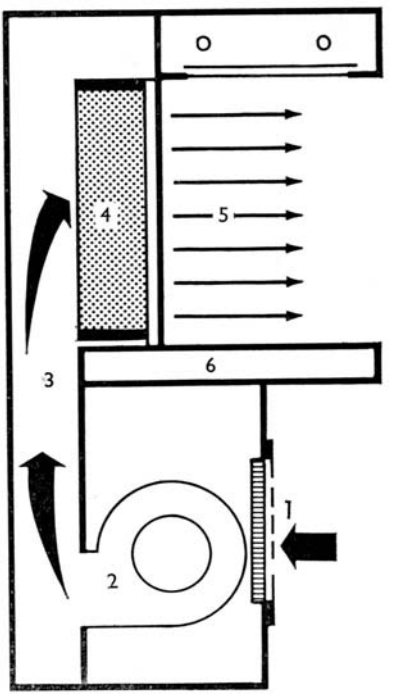

Fig. 1. Diagram of a cross-section of a laminar-flow cabinet with horizontal air flow. 1, Prefilter; 2, blower; 3, pressure box; 4, HEPA filter; 5, sterile area; 6, working table/platform.

The horizontal air flow ('cross-flow') cabinet (Fig. 1) serves to protect material from contamination from outside; vertical air flow in 'down-flow' cabinets, in which a negative pressure is maintained by exhaustion of air under the working area, can be used to prevent contamination of the environment by material in the cabinet (McDade, Sabel, Akers & Walker, 1968; Starzl & Beakly, 1968). Non-turbulent air streams are found to be highly efficient in protecting fine instruments from dust and to improve asepsis in the pharmaceutical and microbiological fields (Favero & Berquist, 1968). McGarrity *et al.* (1969) found that (HEPA-filtered) laminar air flow effectively reduced airborne contamination and factors contributing to the spread of airborne infection. Moreover, clinicians are interested in safe and workable isolation units for nursing patients to be decontaminated with antibiotics (Bodey, Freireich & Frei, 1969; Lidwell & Towers, 1969; Penland & Perry, 1970).

In the present study the protection of germ-free (GF) mice from airborne con-

tamination by laminar air flow was investigated. Both the effect of experimental contamination of the outside air and of the presence of conventional mice inside the clean area of the cabinet were explored.

MATERIALS AND METHODS

Mice

GF mice of the random-bred ND 2 stock and conventional mice of the CBA/Rij strain were used. They were housed in transparent polycarbonate cages (18 × 18 × 12 cm). To reduce the amount of dust produced by the animals, sawdust bedding was replaced by a wire-mesh 1 cm above three sheets of filter paper. The animals were housed two per cage.

Cross-flow cabinets

Four standard type 'clean benches'* manufactured by CEAG (Dortmund, W. Germany) and Bassaire Ltd. (Sussex, Great Britain) were operated with an air velocity in the benches of 50 cm./sec. They were modified by building an entry lock as used in germ-free isolators into one side panel. In addition, two of the cabinets were equipped with a hinged transparent polycarbonate flap as suggested by R. Cook (personal communication). When closed, the flap covers about 95 % of the front side. Two entry ports for long neoprene gloves were built into the flap (Plate 1). To keep the surface and the gloves sterile when the flap was lifted, the flap was divided in two equal parts attached to each other by a long horizontal hinge. In the 'open' position the lower half of the flap covered the upper half completely (Plate 1*B*). The glove entry-ports in the upper half thus became closed by the sterile side of the lower half when the flap was opened and folded. With the flap turned down, the air flow through the cabinet could be reduced to 95 % of its original value; the air velocity in the slit thus became 50 cm./sec. In this way the noise level of the blowers and the risk of dehydration of the animals inside the cabinet could be reduced.

Both the other benches were 'open' and equipped with a wire net to prevent flying insects from entering the clean area.

Down-flow unit

A Bassaire 'portable unit' (with a HEPA filter) was placed on the top of a metal frame to which plastic sheets ending 8 cm. above the floor were fixed to form a tunnel. A standard GF entry-lock and neoprene gloves were built into the side walls to introduce and handle the animals without opening the unit (Plate 2). The cages were placed on a platform 40 cm. above the floor of the room to prevent contamination of the floor surface of the unit. Turbulent air streams in the room in which the unit was placed, caused by opening the doors and walking, resulted in contamination of the floor surface on which the unit was standing.

* Two of these benches have a filter surface of 60 × 120 cm., the others 60 × 180 cm. All cabinets were equipped with HEPA-filters (99·997 % efficiency for particles of > 0·3 μ).

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The effect on the air-stream direction of opening one of the side walls of the flexible plastic tunnel was studied by generating smoke inside the unit. It was found that if one of the side walls was opened, the air stream bends inside the unit above the platform and leaves the unit turbulence-free in a horizontal direction. The air flow at the site of the opening was maintained at 50 cm./sec. The down-flow unit then functioned like an open cross-flow bench and was used accordingly. It was tested with both closed and opened side-wall. In the latter situation the unit was only opened during handling of the animals.

Sterilization of the cabinets and the down-flow unit

After the benches and the down-flow unit had been cleaned and the blowers had been functioning for at least 12 hr., the inside was sprayed with a 2% peracetic acid solution.

In the cabinets with a hinged flap, gloves and flap were wiped with cotton pads soaked in 2% peracetic acid just before the flap was closed. About $\frac{1}{2}$ hr. thereafter GF mice, food, cages and drinking water were introduced from a GF isolator via the entry lock. In experiments with 'open type' benches, GF mice were introduced via the entry lock, and cages with conventional animals through the open front side; they were placed between those containing GF mice. During manipulations inside the sterile chambers of open benches and the down-flow unit, long neoprene gloves (wiped with 2% peracetic acid just before entering the clean area), a face mask and a plastic apron were worn. For obvious reasons the gloves were re-sterilized with peracetic acid between handling the mice of different cages.

Bacteriological investigation of faeces

To investigate whether the GF animals became contaminated, fresh faeces were collected daily from each cage. The faeces were transferred to culture tubes of brain heart infusion broth (Oxoid) and of Brewer's semisolid thioglycolate medium (Difco). These cultures were incubated for 2 weeks at 37° and 21° C. If no growth was observed, the cultures were stirred and subcultured on blood agar plates. These were incubated under aerobic and anaerobic conditions. Faeces were also examined microscopically.

EXPERIMENTAL DESIGN AND RESULTS

In the evaluation of the laminar-flow cabinets, both the protection against contamination from outside and the occurrence of cross-contamination between cages inside the cabinets were investigated.

Contamination from outside

Cages with GF mice were distributed in two rows over the platform of the down-flow unit and the working area of the 'open' and 'closed' cross-flow benches. In the cross-flow benches, cages were also placed on a shelf at the rear end of the working-area half the height of the filter surface (Plate 1A). In the 'closed'

benches the flap was opened only during sampling of the faeces. In this type of cabinet cleaning of cages, etc., was performed with the gloves built into the flap.

The down-flow unit was tested for two periods of 6 weeks. In the first period, sampling and handling were done with the gloves built into the unit. The unit was never opened during this period. In the second 6-week experiment the unit was used in the manner described for the 'closed type' bench. Sampling was performed with long sterile gloves after one of the side walls was opened and the air velocity had been adjusted accordingly.

In the first week of all experiments, the air in front of the air inlet filter was contaminated twice by nebulizing suspensions of *Escherichia coli*. These suspensions were also nebulized in front of the open type benches. In each case 10 ml. of suspensions containing 5×10^9 organisms/ml. were nebulized in about 30 sec.

Two weeks after introducing the GF mice the blowers of the 'open type' cabinets were switched off. The bacteriological investigation of the animals was then continued for a few days to see whether contamination from outside occurred.

Results

The experiments in the down-flow unit were very satisfactory. During their 3 months stay in the unit none of the mice became contaminated. In the cabinets with a hinged flap no cage was found to be contaminated during an observation period of 2 weeks.

Results with the 'open' cabinets were different. Animals in cages directly in front of the filter remained sterile. However, cages in the front row, located near the open side, were found to be contaminated after various intervals. After the blowers had been switched off, all the cages became positive within 2 days. This shows that the laminar flow of sterile air had been essential in protecting the area in front of the filter panel from contamination from outside.

Cross-contamination

In this type of experiment a stream of non-turbulent air of sufficient velocity between the cages is critical. Therefore only the 'open type' benches were used. The air velocity was maintained at 50 cm./sec. Four cages with GF mice were placed directly in front of the filter wall at a distance of about 32 cm. from each other. Cages with conventional mice were then placed between them. The cage distance thus became about 10 cm. After 1 week the distance was reduced to 7 cm., after 2 weeks to 5 cm. and in the third week to 3 cm.

Results

In four identical experiments no cross-contamination occurred in the first week when the cages distance was 10 cm. When the cage-distance was reduced to 7 cm. in the second week, one cage became contaminated in one of the four experiments. The contaminated cage was then replaced. In the third week most cages became positive and were also replaced. When the cage distance finally was 3 cm., all cages were found to be contaminated within a few days. From these results it was evident

that cage distance was a predominant factor in protecting from cross-contamination. When the importance of cage distance was realized, three 'open type' cross-flow benches could be used during the past year in antibiotic decontamination experiments in mice without any spread of contamination.

DISCUSSION

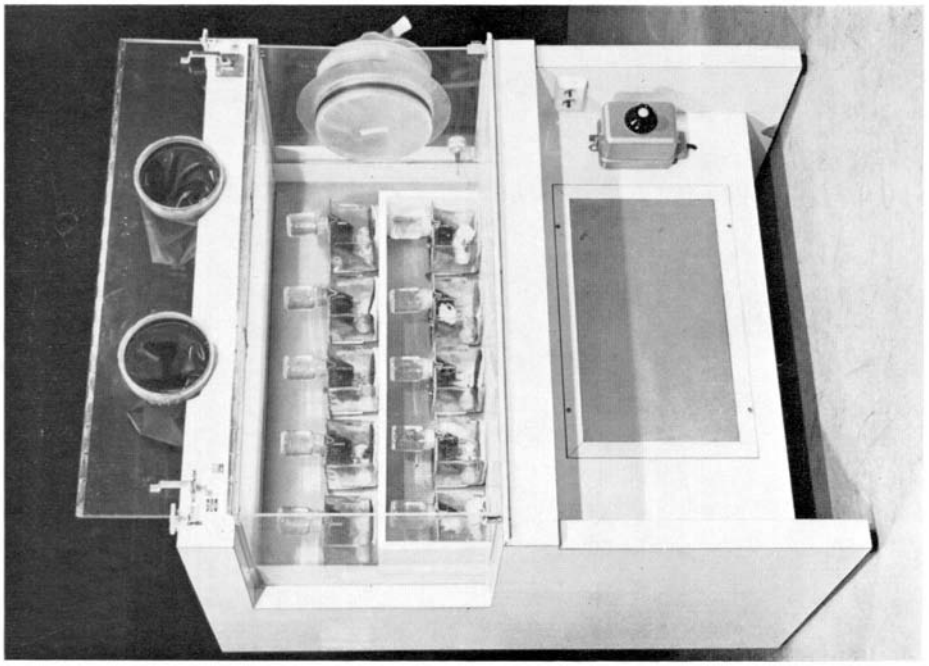
It can be concluded from the results reported that laminar-flow cabinets equipped with HEPA air filters and modified in the way described can afford adequate protection against airborne contamination for a short period of time. In the 'open type' benches the protected area is limited to the rear of the working area directly in front of the filter panel. The cabinets modified with a hinged flap, however, gave protection against airborne contamination from outside over the entire working area. Cages located in the front row also remained free from bacterial contamination. For this reason the 'closed type' benches are preferable to 'open' benches when only prevention of contamination from outside is sought. Accordingly, they have been used in our Institute for short-term surgical experiments in germ-free mice.

The successful isolation provided by the 'open type' benches could have been positively influenced by the fact that the bacterial concentration of the air in the room was low. This was because all four cabinets were located in the same room and the air of that room was continuously recirculated through the cabinet filters. With a slit-sampler, between 5 and 50 colony-forming units were found per 500 l. of air. In a comparable room without cabinets the bacterial concentration was ten time higher.

The down-flow unit described and tested in this study also provided adequate protection from airborne contamination during 3 months. It is stressed, however, that neither the down-flow unit nor the 'closed type' cabinets can (in their present form) replace the standard type of GF isolator. The benches, for example, do not prevent ants and other small insects from entering the sterile chamber. The 'laminar-flow isolator' (closed type, benches and the down-flow unit) may, nevertheless, find useful applications in some short-term experiments.

In the 'open type' benches the area directly in front of the filter panel was not only protected from contaminations from outside, but also from cross-contamination between cages. The latter depends, however, on the cage distance. No cross-contamination from cage to cage occurred, if the air stream could pass between the cages over a distance of at least 10 cm. After the benches were tested with GF and conventional mice they were used in decontamination experiments. No cross-contamination has been seen since. So far, mice were decontaminated in standard laboratory racks closed at the sides with sheets of plastic (van der Waaij & Sturm, 1968). Although airborne gross contamination was limited in this way, contaminations with antibiotic-resistant bacteria were seen from time to time under these isolation conditions.

On the basis of experiments performed with animals in the 'closed type' benches, a cross-flow cabinet with a hinged flap was used with good success for



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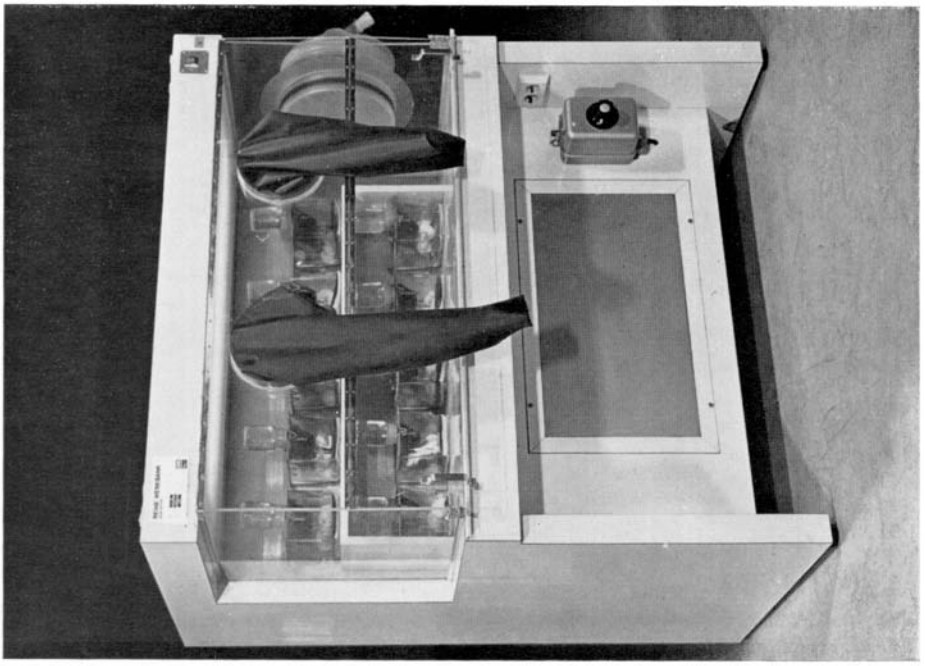

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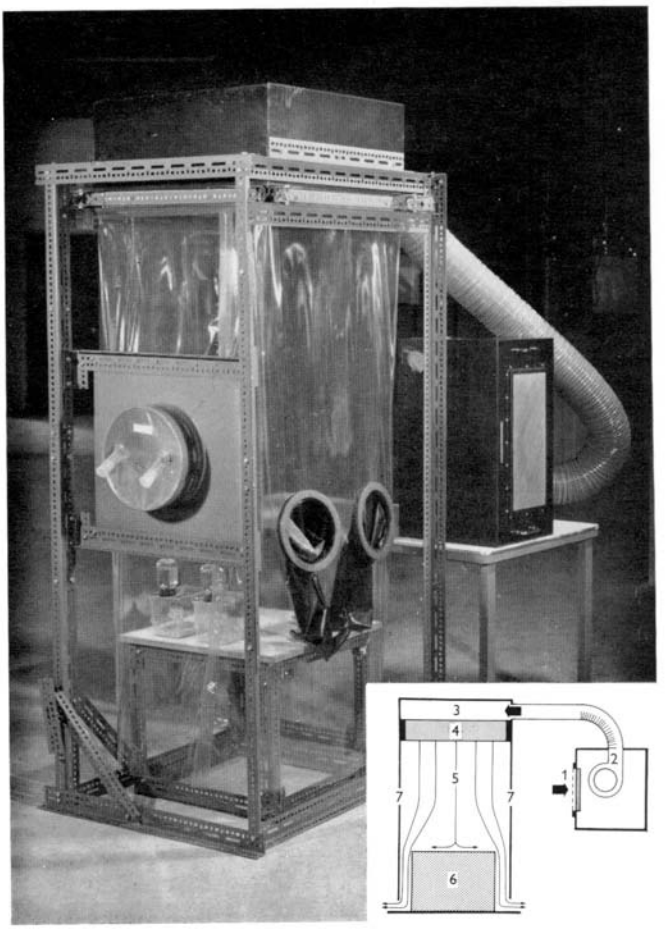

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clinical purposes. A human baby suffering from congenital Swiss-type agammaglobulinaemia has been isolated for 6 months in such an isolation system (de Koning *et al.* 1969). Furthermore, from the results obtained with the down-flow unit, it was concluded that a down-flow unit with a raised floor area (to be used only by the patient) can be recommended for strict isolation of human patients. A unit of this type has been in use now in the paediatric department of the Medical School of Rotterdam for a period of over 8 months for the isolation of a 12-year-old boy. This boy was isolated and (antibiotic) decontaminated because of heavily infected *Pemphigus vulgaris* lesions of the skin and oral mucosa.

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EXPLANATION OF PLATES

Plate 1

A, Laminar-flow cabinet modified with a hinged flap with gloves and GF-type entry-lock in the side wall. B, Laminar-flow cabinet with an 'opened' hinged flap (note that the lower half of the flap closes the glove entry ports; in this way the inner side of the flap and the gloves are maintained sterile).

Plate 2

Down-flow unit with a raised floor, a GF entry-lock and long neoprene gloves, built into the side wall for handling. 1, Prefilter; 2, blower; 3, pressure box; 4, HEPA filter; 5, sterile area; 6, working table/platform; 7, flexible plastic side walls.