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Physical protection against airborne pathogens and pollutants by a novel animal isolator in a level 3 containment laboratory

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SUMMARY

A containment laboratory unit for research with aerosols of group 2 pathogenic microorganisms is described. The design criteria are based on current UK guidelines, which imply containment at group 3 level during aerosol production, storage, exposure of animals and sampling. Within the aerosol laboratory, primary containment is provided by a Henderson apparatus operating at a negative pressure to the external environment. Flexible film isolators under negative pressure are used for all hazardous microbiological work, e.g. tissue homogenization, and for housing infected laboratory rodents. A novel feature of the animal isolator is the separate ventilation of each cage, which minimizes the risk of cross-infection by aerosol transmission and ensures a similar environment within each cage. The results of an intentional release of a cloud of non-pathogenic microorganisms are presented to show the effectiveness of the containment barriers. Recommendations are given for the safe operation of a containment unit based upon practical experience.

INTRODUCTION

Research in aerobiology often involves the deliberate production of aerosols of pathogenic microorganisms and allergenic or toxic dusts which may then be used in studies of microbial survival, pathogenicity or toxicity. Aerosols can also be generated accidentally during routine experimental procedures and avoidance of their creation is one of the principal tenets of good laboratory practice. Containment of aerosols of either sort is necessary (i) to protect research workers against inoculation by inhalation or other routes; (ii) to reduce the risk of cross-transmission of pathogens between animals infected experimentally; and (iii) to avoid accidental release to the environment.

Interest in aerosol containment has developed from a number of standpoints. Official concern in the UK over laboratory-acquired infections culminated in the establishment of the Advisory Committee on Dangerous Pathogens [1] and the publication of a model code of conduct for work with pathogenic microorganisms ([1]; see [2] for a full account). Cross-transmission of pathogens is an avoidable hazard in experimental infections and is best prevented by separate ventilation of

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individual animal cages [3], although the practice is uncommon because of limited necessity for this degree of control. More recently, the role of aeroallergens has been recognized in respiratory allergies to laboratory animals among animal technicians [4] but feasible engineering solutions to reduce aerial concentrations have not yet been devised.

This paper describes the design, construction and performance of a new containment laboratory unit for research with aerosols of hazard group 2 pathogens. The unit incorporates a novel flexible-film isolator for housing infected animals, which not only minimizes the risk of cross-transmission of pathogens between cages but also maintains a similar physical environment within different cages and reduces greatly the release of aeroallergens and pathogens.

DESIGN AND CONSTRUCTION

Containment laboratory unit

The major design requirement was a laboratory unit in which aerosols of hazard group 2 pathogens could be generated intentionally for studies of microbial survival in air and of the fate of inhaled microorganisms in the respiratory tract of laboratory animals. Accidental exposure to aerosols is part of hazard assessment of work with human pathogens but explicit guidance is not given where aerosols are generated intentionally [1]. However, the ACDP code recommends that 'the level may be altered to provide more stringent containment conditions depending upon the local assessment of risk'. Accordingly the decision was taken to provide level 3 containment when aerosols of hazard group 2 were created intentionally. The main experimental procedures occurring in the unit are aerosol production, storage and sampling, inoculation and dissection of laboratory animals, and tissue homogenization.

The unit was designed jointly by the authors and M. Walden of Fell Clean Air Limited, Newhaven, Sussex. Figure 1 shows the plan layout of the unit and Figs. 2 and 3 show the aerosol laboratory and animal isolator respectively. The unit is divided into four main areas and the layout was determined in part by an existing stanchion in the building's centre. The service lobby provides entry to all laboratories and has restricted access. All work involving aerosols is carried out within the aerosol laboratory of containment level 3 using a mobile Henderson apparatus [5] connected to either a 75 l rotating drum [6] or a mouth-and-nose only inoculation chamber for 54 mice [7]. The Henderson apparatus provides an air recirculation system operating under negative pressure with several in-line high efficiency particulate filters (HEPA) and accurate control of relative humidity. Primary containment of aerosols is provided by the Henderson apparatus but pressure-ventilated hoods (RH-17810/2, Martindale Protection Ltd, London) are worn at all times as a secondary precaution against leakage or explosion. Entry to the aerosol laboratory is via a combined shower/airlock with an electric door interlock to prevent simultaneous opening of both doors. Microbiological work is undertaken either on the open bench in the aerosol laboratory or, for hazardous procedures such as tissue homogenization, in a six-glove flexible film isolator (Cambridge Isolation Technology Ltd, Cambridge) in the microbiology laboratory (containment level 2). The microbiology isolator also allows transfer of materials, animals and equipment between the aerosol

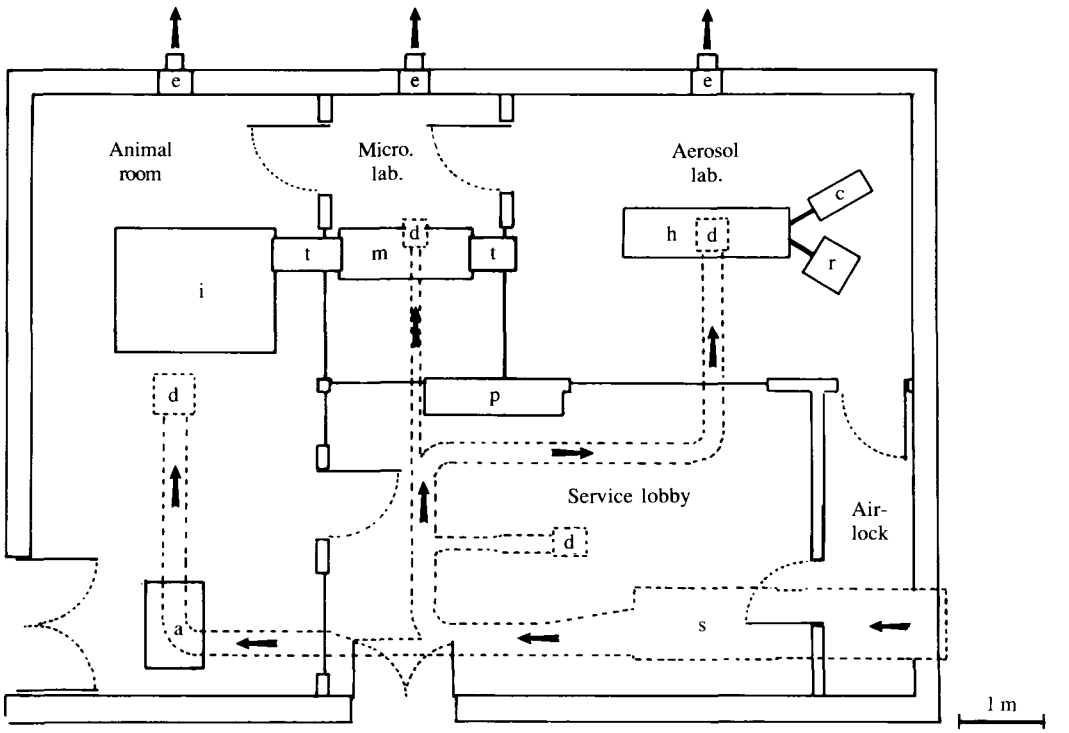

Fig. 1. Plan layout of the containment laboratory unit: a, autoclave; c, inoculation chamber; d, diffuser; e, exhaust fan and filters; h, Henderson apparatus; i, animal isolator; m, microbiology isolator; p, control panel; r, rotating drum; s, supply fan and filters; t, transfer chamber. Arrows show direction of the airflow in ventilation ducts (shown dashed).

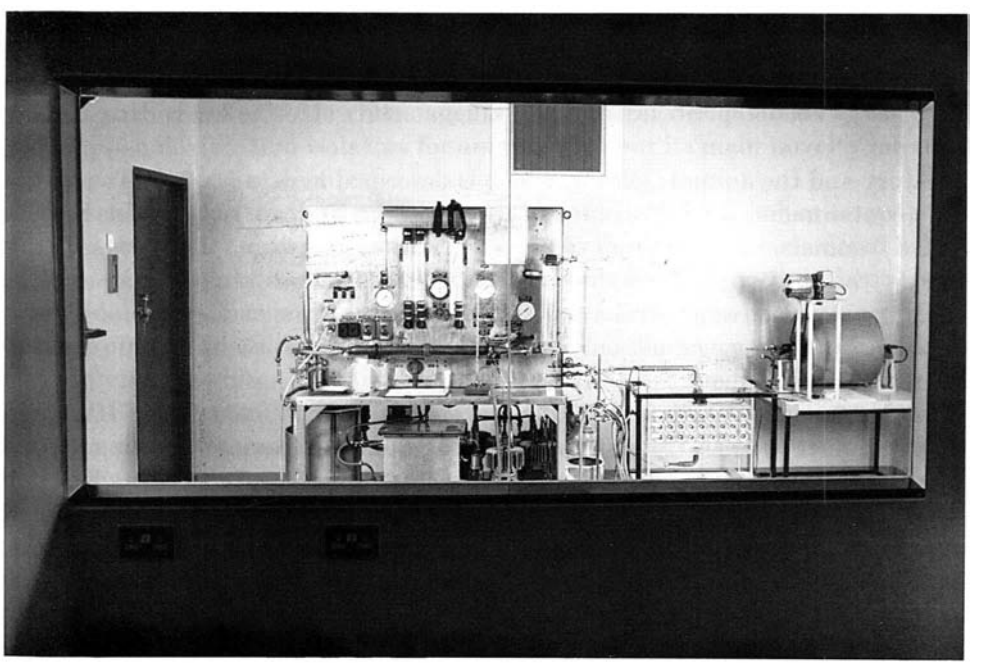

Fig. 2. View of aerosol laboratory from service lobby.

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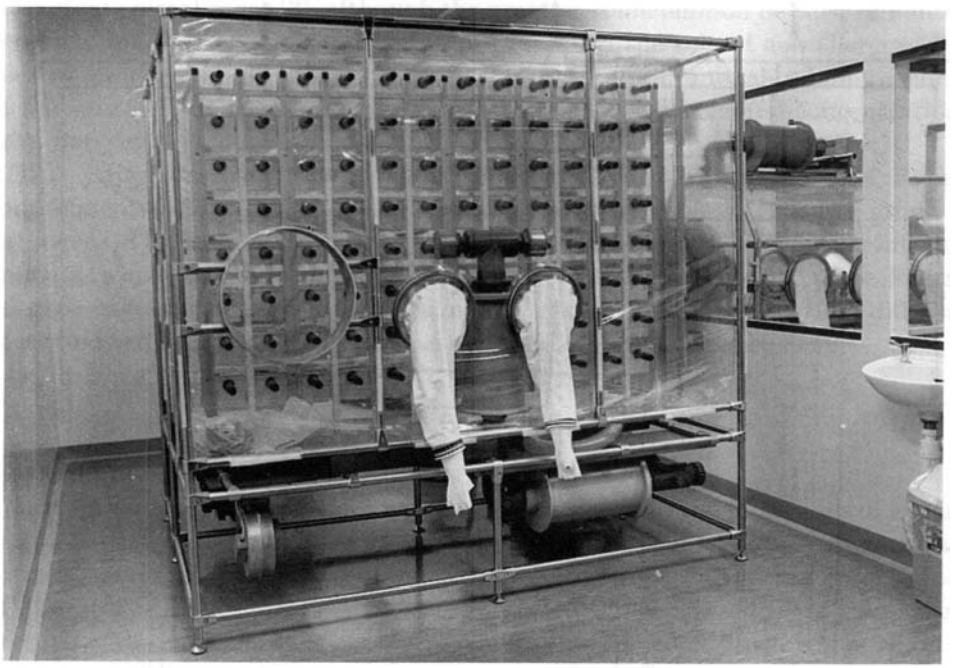

Fig. 3. View of animal isolator (foreground) with microbiology isolator seen at rear.

Table 1. *Ventilation specifications for an aerobiology containment laboratory unit*

	Containment level	Ventilation rate (air changes/h)	Pressure differential (Pa)
Service lobby	2	7·7	0 wrt atmosphere
Animal room	2	15	−10 wrt atmosphere
Microbiology laboratory	2	15	−10 wrt atmosphere
Aerosol laboratory	3	15	−20 wrt atmosphere
Animal isolator	3	30	−30 wrt room
Microbiology isolator	3	15	−30 wrt room

laboratory and the animal isolator, which is described in detail below. The animal room (containment level 2) contains an autoclave and an isolator for housing infected animals. A three-way transfer chamber between the two isolators provides the only portal to the isolator system. Access to the microbiology laboratory is via the animal room, but if this entrance from the service lobby were to be sealed and entry gained only via the shower/airlock, then the unit could be operated at containment level 3.

Specifications for ventilation (Table 1) were determined from the ACDP model code of conduct [1], the Home Office code of practice for animal housing [8] and the British Standard for environmental cleanliness in enclosed spaces [9]. The unit is ventilated mechanically without recirculation. Fresh air is supplied via two coarse filters by a single fan (Fischbach) and is ducted to each of the main areas; approximately 20% of the supply is admitted via pressure relief flaps which can be adjusted to balance the relative airflows and also extract air from the service lobby. Air is exhausted to atmosphere via a coarse and HEPA filter (0·003%

penetration at 0·3 μm) by one of three fans (Sifan) mounted in the end wall. The speed of each fan is controlled separately to allow for changes in filter resistance. Minimum air change rates and pressure differentials are not specified for containment level 3 animal rooms and laboratories in the ACDP code [1] and so standards for clean rooms were adopted [9]. Minimum rates of 15 (room volume) air changes per hour (a.c./h) were specified for the areas of greatest risk and of 7·7 a.c./h for the service lobby. Pressure differentials of −10 and −20 Pa with respect to atmosphere were specified for the animal room and microbiology laboratory and for the aerosol laboratory respectively: the ACDP code specifies a differential of −69 Pa for a containment level 4 laboratory. The supply and exhaust fans are interlocked to prevent positive pressurization of the laboratories in the event of failure of the extract fan(s). Twin back-up fans and a standby generator could have been installed but the additional expense was not considered worthwhile, except for the animal isolator. Reverse airflows are prevented by the pressure relief valves mounted in the walls and doors. The unit is heated electrically by main and subsidiary batteries in the air supply ducts. A small temperature difference of about 5 °C can be maintained between the main areas, each of which has its own thermostat. There is no provision for either cooling or control of humidity.

The unit is located in an existing building. Internal dividing walls were constructed from metal stud partition with two layers of gypsum board enclosing a 100 mm cavity. Internal windows of 6 mm polished glass plate were installed from dado to 2·1 m height in all internal walls to promote safe, friendly working conditions. The walls and ceiling were sealed with two coats of epoxy paint.

Animal isolator

The animal isolator was designed jointly by the authors and T. Coles and J. Tombs of Cambridge Isolation Technology Ltd, Cambridge and is the subject of a pending patent application [10]. It is used currently to house mice infected by aerosols of hazard group 2 pathogens, but its containment is suitable for hazard group 3 pathogens or toxic chemoagents. Its general design follows the ACDP guidelines on flexible film isolators for animals [11] but its main novelty lies in the separate ventilation of each cage.

The isolator is shown in Fig. 3 and in end profile in Fig. 4. It has a capacity of 96 M1 cages, each capable of holding five mice. Each cage is considered to be a separate experimental unit and therefore one mouse only is usually kept therein to minimize use of experimental animals. The isolator operates at a negative pressure of −30 Pa with respect to atmosphere. Laboratory air is drawn by an inlet fan via a pre-filter of polyether foam and HEPA filter (0·003% penetration at 0·5 μm) and distributed by a perforated PVC plastic duct to the front chamber. Air is then drawn through the top half of each cage individually via a gauze pad and exhausted to a plenum chamber via a spigot of 17 mm minimum internal diameter and 114 mm length. The spigot passes through an undersized hole in the back wall of the plenum chamber and thereby maintains a good seal. Air is extracted from the plenum chamber via two pre-filters in parallel and a primary exhaust HEPA filter (specifications as above) in series, all mounted in the chamber. A secondary exhaust HEPA filter mounted externally is placed in series

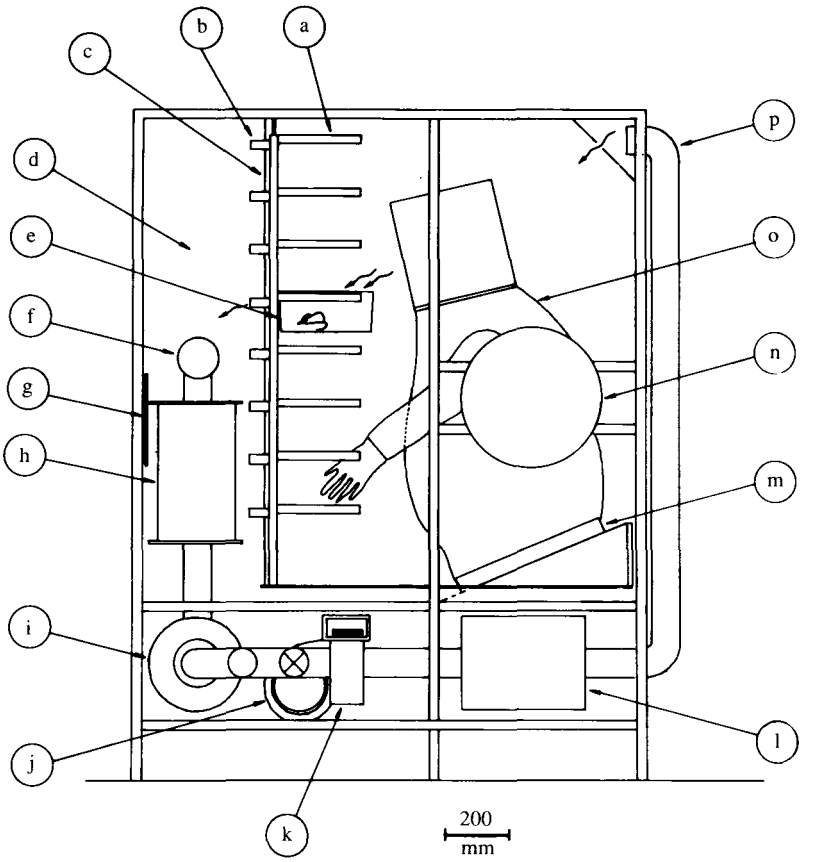

Fig. 4. End profile of the animal isolator: a, cage rack; b, exhaust spigot; c, PVC membrane; d, plenum chamber; e, mouse cage; f, exhaust prefilter; g, jampot; h, primary exhaust HEPA filter; i, secondary exhaust HEPA filter; j, inlet fan; k, exhaust fan; l, inlet HEPA filter; m, half-suit base; n, three-way transfer chamber; o, half-suit; p, air inlet. Arrows show the direction of airflow through the isolator.

with the primary filter. The speed of both inlet and exhaust fans can be controlled independently to adjust the ventilation rate and canopy pressure differential between the isolator and the animal room. Ventilation rate is measured indirectly by the pressure drop across an orifice fitted in the exhaust side and the differential is measured directly by an inclined manometer. An alarm is triggered when the differential falls below -15 Pa. The ventilation rate is usually set to 90 m^3/h, which, with due allowance for the volume of the half-suit, corresponds to about 30 canopy volume a.c./h and about 200 cage volume a.c./h. The pressure differential and ventilation rate both exceed the minima set by ACDP [11].

PERFORMANCE

Containment laboratory unit

The performance of the unit has been satisfactory since its completion in June 1989. A minimum of two, and preferably three, staff are needed during aerosol

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inoculation of mice. Air pressure differentials and temperature are monitored daily and weekly respectively and exhaust fan speeds require adjustment every fortnight. The greatest potential for contamination of staff arises during movement of mice from the inoculation chamber to the transfer port in the microbiology isolator. In operation, the inoculation chamber is purged for 10 min with an aerosol of distilled water after the animals have been exposed to the bacterial aerosol to minimize aerosol release to the laboratory. This hazard could be eliminated by enclosing the chamber in a purpose-built flexible film isolator attached to the transfer port. Similarly, enclosure of the entire Henderson apparatus would remove the need for personal respirators, though the design of an appropriate isolator would be more difficult.

The integrity of the suite was tested following a simulated accidental release of aerosol from the Henderson apparatus to the aerosol laboratory. Microbial aerosols are normally generated by a triple-jet Collison nebulizer [12] containing 10 ml of suspension fluid at a concentration of 10^9 c.f.u./ml and operated at an airflow rate of 8·4 l/min at 180 kPa. The maximum release to the laboratory from a discharge of the entire contents of the suspension vessel would correspond to an airborne concentration of 2×10^8 c.f.u./m^3 if there was no sedimentation, dilution by ventilation or death *in situ*. In the simulation about 10^{10} c.f.u. of *Bacillus subtilis* var *niger* was discharged from a triple-jet Collison nebulizer operating as above.

Two tests were run: (i) with the ventilation system fully functional; and (ii) with the system disabled, as would occur following a power failure. The discharges lasted 4 and 10 min respectively. The aerosol concentration was measured by raised Porton all-glass impingers placed centrally in each room at 0·9 m height. Impingers contained 10 ml peptone saline and were run for 10 min, commencing at the start of the deliberate discharge. Impinger collecting fluids were diluted serially in peptone saline and viable counts determined in quadruplicate on nutrient agar plates after overnight incubation at 37 °C. Settle plates were also placed at the same sites and on the floor at the doorway between rooms. In the second test another set of samples were collected 1 h after the discharge to allow time for dispersion of the cloud throughout the unit. Even though the aerosol samplers were operated remotely from the service lobby, entry to each of the rooms was necessary to change the impingers between the first and second set of samples.

Tables 2 and 3 present the results of the integrity tests. The airborne concentration of *B. subtilis* in the aerosol laboratory reached $3{\cdot}5 \times 10^5$ and $1{\cdot}68 \times 10^6$ c.f.u./m^3 during the first 10 min after the start of the discharge in both the first and second tests. In contrast, the aerosol concentrations in the other rooms were very low when the ventilation system was operating normally, but their measurements were subject to inaccuracy since the impinger fluid concentration was typically 2 c.f.u./ml, i.e. close to the detection limit. The colony counts on the settle plates were also very low. A protection factor was calculated from the total discharge to each room, i.e. the product of aerosol concentration and room volume, and ranged from 1400 to ∞.

Switching off the ventilation system (to simulate power failure) caused an immediate dispersal of organisms, especially into the microbiology laboratory and

Table 2. *Airborne concentration of* B. subtilis *after a simulated accidental release from the Henderson apparatus in the aerosol laboratory*

		Ventilation functional		Ventilation failure			
				at release		1 h later	
Location	Volume (m^3)	C (c.f.u./m^3)	P	C (c.f.u./m^3)	P	C (c.f.u./m^3)	P
Aerosol laboratory	50	$3{\cdot}50 \times 10^5$	—	$1{\cdot}68 \times 10^6$	—	$4{\cdot}98 \times 10^6$	—
Microbiology laboratory	21	0	∞	$2{\cdot}12 \times 10^4$	189	$4{\cdot}01 \times 10^5$	29
Animal room	73	164	1470	$5{\cdot}87 \times 10^2$	1960	$3{\cdot}90 \times 10^4$	87
Shower/airlock	11	105	15100	$6{\cdot}40 \times 10^4$	119	$5{\cdot}27 \times 10^5$	43
Service lobby	64	194	1410	95	13800	$4{\cdot}83 \times 10^4$	80

C, Concentration,

$$\text{P, protection factor} = \frac{\text{total discharge in aerosol lab.}}{\text{total discharge in the other location}}$$

Table 3. *Settle plate counts of* B. subtilis *after a simulated accidental release from the Henderson apparatus in the aerosol laboratory*

		Ventilation failure	
	Ventilation functional	at release	1 h later
Aerosol laboratory	3	4	4
Aerosol lab-microbiology lab door	0	4	4
Microbiology laboratory	0	3	3
Microbiology lab-animal room door	1	1	2
Animal room	1	1	2
Animal room-service lobby door	2	1	2
Aerosol lab-shower/airlock door	1	4	4
Shower/airlock	1	3	4
Shower/airlock-service lobby door	1	2	3
Service lobby	1	0	2

Categories: 0, 0 c.f.u./plate; 1, 1–9 c.f.u./plate; 2, 10–99 c.f.u./plate; 3, 100–999 c.f.u./plate; 4, > 1000 c.f.u./plate.

shower/airlock and thus a substantial fall in the protection factors (Table 2). The protection factors declined further 1 h after the discharge had ceased and ranged between 29 and 87, with the highest values recorded in the service lobby and animal room. The settle plate data showed a similar pattern of tracer spread over time and throughout space.

Animal isolator

The performance of the isolator was assessed in terms of the cross-transmission of pathogenic bacteria between cages, the uniformity of the physical environment between cages and its overall suitability for keeping laboratory animals.

The isolator has been used continually since installation for over a year. During

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this period six experiments involving 532 mice have been conducted on the infectivity of 15 strains of *Klebsiella pneumoniae*. The duration of each experiment ranged from 9 to 49 d including a minimum period of 7 d of acclimation to the isolator. In each experiment mice were inoculated by an aerosol generated in the Henderson apparatus, then returned to the isolator and caged individually. At various intervals mice were killed to obtain cultures of *K. pneumoniae* from lung homogenates. Uninfected control animals, kept in separate cages positioned at random, were treated analogously but failed to provide any positive isolates during this period. Furthermore, in some experiments several strains of *K. pneumoniae* were tested simultaneously in separate groups of mice but, again, there was not any evidence of cross-transmission of different strains between cages.

The uniformity of the physical environment was tested by measurements of air velocity at the exhaust spigot and of air temperature and concentration of ammonia within representative cages. All measurements were taken at nine locations spaced uniformly across the rack, i.e. on the first, fourth and eighth rows. Air velocity was measured with a hot-wire anemometer at the centre of the exhaust spigot at two ventilation rates, nominally 50 and 90 m^3/h, described as low and high respectively. The overall mean ($\pm$S.D.) air speed was 0·54 ($\pm$0·062) and 0·85 ($\pm$0·062) m/s ranging from 0·5 to 0·65 and from 0·75 to 0·95 m/s. Assuming an effective spigot diameter of 20 mm such speeds correspond to cage ventilation rates of 140 and 210 a.c./h. Even though these rates indicate a high air turnover, the velocity measured directly beneath the intake gauze pad was below 0·1 m/s.

Air temperatures beneath the gauze pad intake within cages were measured simultaneously with thermistor probes at 5 min intervals for 1 h at both ventilation rates and at an ambient air temperature of 23 °C. The mean temperature difference between cage and ambient temperature is shown in Fig. 5. The overall mean was 0·18 °C with means ($\pm$S.E.) for individual cages ranging from +0·02 ($\pm$0·021) to +0·60 ($\pm$0·048) and from −0·06 ($\pm$0·033 to +0·63 ($\pm$0·038) °C at low and high ventilation rates respectively. The maximum difference was recorded at location y which is adjacent to the base of the half-suit. Data were analysed by a four-way analysis of variance with vertical and horizontal coordinates treated as three-level factors nested within the effects of ventilation rate and with time treated as a blocking factor. This analysis indicated a highly significant interaction ($P < 0·001$) between the vertical and horizontal coordinates which depended on ventilation rate. The standard error of the difference between means for this three-way interaction was 0·056 °C which indicates the high sensitivity of the experimental design.

Ammonia concentrations were measured close to the bedding in each cage with Draeger diffusion tubes over an 8 h period. All measurements were below the limit of detection of 2·5 p.p.m.

The husbandry of the mice in the animal isolator has been most satisfactory to date. In an earlier design of cage top the gauze pad extended over the full length of the cage but the mice devoured that part of the pad in proximity with the cage mesh, thereby altering the airflow pattern. The prefilters in the exhaust were replaced daily, though the life expectancy of the HEPA filters is estimated to be

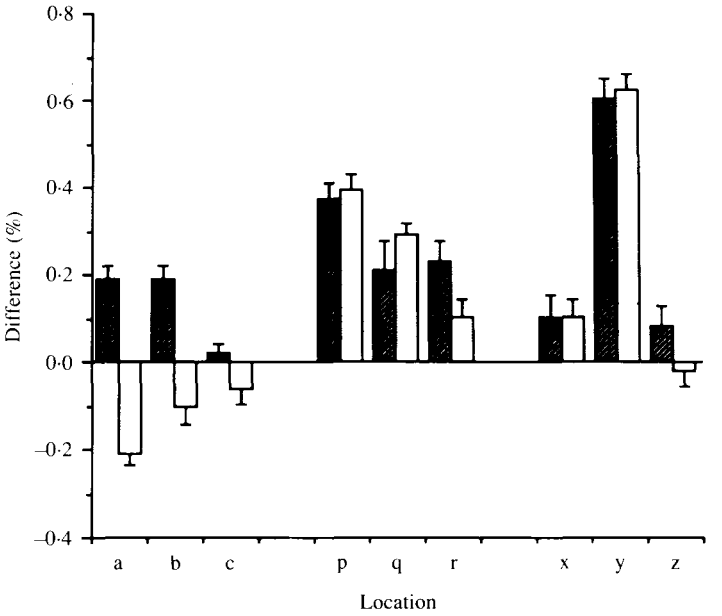

Fig. 5. Air temperature uniformity in the animal isolator (▨, low; □, high ventilation rate) expressed as the mean (+S.E.) difference between cage and isolator. Locations (a, b, c), (p, q, r) and (x, y, z) refer to three cages spread across the first (top), fourth and eighth row respectively.

at least 3 years. Other aspects of operation are similar to those of conventional flexible film isolators for laboratory animals.

DISCUSSION

Infection by aerosols has long been recognized as a major hazard in work with pathogenic microorganisms. Many common microbiological techniques, e.g. pipetting, plating cultures, centrifugation and homogenization, may produce respirable aerosols either through accident or misuse [2]. Protection against aerosol infection of workers and the environment is usually on the basis of a three-barrier system [13].

In our unit the major hazards arise in the aerosol laboratory in which the Henderson apparatus provides the primary barrier, relying for its safety on a negative air pressure differential, automatic cut-off of the compressed air supply to the aerosol generator and HEPA filtration (5). A secondary barrier is needed in the event of failure of the primary barrier and it often takes the form of a safety cabinet, the laboratory itself or a personal respirator. Enclosure of the Henderson apparatus, rotating drum and inoculation chamber would be preferable – but more costly and inconvenient – to the current reliance on respirators; furthermore, the protection factor could be greater. The public at large and the environment usually are protected from accidents by tertiary barriers which often provide environmental control for experimental equipment [13]. In our unit this barrier is the laboratory itself, complete with its safety fixtures such as mechanical ventilation and exhaust air filtration.

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There is a surprising lack of recommendations for minimum air change rates and air pressure differentials in containment laboratories (e.g. [2]) in contrast to the exacting specifications for clean rooms [9], which for the greater part are mirror images of the former. Neither parameter is specified by ACDP for level 3 containment, though a minimum pressure differential of −69 Pa is given for level 4 laboratories [1]. The Home Office code of practice states that '15–20 changes of fresh or conditioned air per hour distributed throughout the room are normally adequate for rodents and lagomorphs' [8], though it may be technically difficult to ensure a uniform distribution without stagnant zones in a large space, especially where animals are kept in cages with solid floors and walls. The minimum ventilation rate therefore was set at 15 a.c./h, a value which recognizes a compromise between rapid dilution in the event of slow leakage or an accident and excessive costs of heating and filtration. Similarly, the pressure differentials of 10 Pa are small – 10^{-4} of an atmosphere – and rely on well-fitting doors and windows with minimal adventitious infiltration.

There are few published accounts of a simulated accident involving discharge of a microbial aerosol within a containment laboratory. Spore clouds are a recommended alternative to sodium chloride or dioctyl phthalate aerosols for testing safety cabinets [2] and flexible film isolators [11]. *B. subtilis* was chosen as a test organism because its aerodynamic characteristics are similar to those of the vegetative bacteria usually used in our experiments. The first test recorded a protective factor in the rooms adjacent to the aerosol laboratory of at least 1400 (notwithstanding the inaccuracy of the estimates arising from the low plate counts) and demonstrated the effectiveness of both the air pressure differential and rapid ventilation rate. These estimates of protection factors were lower than anticipated *a priori*. Part of the explanation lies in the possible transfer of tracer by a 'pumping action' of the doors during exit from the aerosol laboratory. Remote operation of the Henderson apparatus or change of the samplers was not feasible. Consequences of a failure of the ventilation system coincident with a sudden discharge of aerosol, as simulated in the second test, were grave and demonstrate a significant hazard, especially after the original cloud had dispersed throughout the unit for 1 h.

Workers within the aerosol laboratory face two distinct hazards. First, minor leaks of aerosol can arise during replacement of impinger samplers or removal of infected mice from the inoculation chamber, though purging of the system with an aerosol of distilled water decreases the latter hazard. Chatigny and Clinger [13] estimate for the Henderson apparatus that about 0·1 % of the total volume of about 5 l may escape to atmosphere in such procedures, thereby releasing about 5000 microorganisms for an aerosol concentration of 10^6 c.f.u./l, but whether this would be a serious hazard depends on the human infective dose and degree of protection. Good personal respirators provide a protection factor of at least 10^5 and therefore such minor leaks are of little consequence, even for low infective doses.

The second hazard to the worker is potentially more serious. In the unlikely event of complete aerosolization of the contents of the suspension vessel about 10^{10} microorganisms would be discharged. Between 10 and 90 % of microbes, depending on species, would not survive the stresses of aerosolization [14] while others would

disperse through the room volume within several minutes. Hence workers could be challenged with an aerosol concentration of *c.* 10^8 c.f.u./m^3 which, for a protection factor of 10^5 and inhalation for 10 s at a minute volume of 6 l, represents a dose of a single microorganism. More importantly the combined result of an accidental release of aerosol from a Henderson apparatus and a simultaneous failure of the ventilation system would quickly contaminate the containment suite. The airborne concentration of microorganisms in the animal room and service lobby then may be expected after 1 h to reach about 1/80 of that in the aerosol laboratory, whence a serious hazard could arise for unprotected personnel. Prompt action would be needed. Workers within the aerosol laboratory, who would be protected by personal respirators, would release a chemical fumigant to sterilize the contaminated area. They would not leave the laboratory until unprotected personnel in other rooms had either left the unit or donned personal respirators, because movement through the unit would enhance dispersal of aerosol. These simple calculations reinforce the need in aerobiological research for effective maintenance of aerosol apparatus and ventilation systems, careful laboratory techniques and high efficiency respirators.

Our results indicate that the benefits of individual ventilation of animal cages are twofold. First, the risk of airborne transmission of pathogens is diminished greatly and, second, the climate within the cage is similar throughout the cage rack. [Protection of the worker from airborne allergens, pathogens and toxic chemoagents is, of course, an additional advantage of isolators in general. It applies equally to a simple development of the current design of ventilated cage which has a more widespread utility (Wathes, Johnson, Coles and Tombs, unpublished).]

Early versions of ventilated cages usually employed an exhaust manifold with flexible hose connections [3] while alternative forms of ventilation included a filter rack [15] (subsequently shown to be ineffective [16]), a laminar flow system [17], a mass airflow system [18] and a positive pressure manifold with a perforated supply pipe [19]. Our system has similarities with that developed by Jenkyn, Hirst and King [20] for the isolated propagation of foliar pathogens in that both utilize a large volume plenum chamber to ensure uniform ventilation rates between units and have a rapid air change rate, *c.* 31 a.c./h in the latter. However, our design of a perforated PVC membrane for sealing the spigot to the plenum chamber is apparently novel: it is also simple but effective. The length of the spigot and the flexibility of the membrane allow some latitude in location of the cage.

Provision of a uniform environment across a rack of animal cages has been the goal of many workers since non-uniformity introduces avoidable variation in biological responses [8]. For example, Clough [21] showed a temperature difference of 4·9 °C between the top and bottom rat cages of a rack in an animal room with an apparently efficient air distribution system and a rapid (room) ventilation rate of 17 a.c./h. Though differences in cage temperature were statistically significant in our trial, in practice they are small enough to be of little biological relevance. The Home Office code of practice specifies an optimal room temperature range of 19–23 °C for mice while acknowledging that the temperature within the cage will be higher [8], yet one comprehensive study showed that laboratory mice can tolerate a wider band if the acceptance criterion are based on growth rate.

reproduction, haematological parameters and organ weights [22]. This conclusion is probably not appropriate for other responses, e.g. activity of pharmacological or toxic agents which are sensitive to small changes in metabolic rate resulting from the maintenance of homeostasis.

In conclusion, the development of flexible film isolators has enabled new approaches to protection against airborne pathogens and pollutants to be used in aerobiological research. Their major advantages over conventional rigid isolators are a flexible design and simplicity of construction which more than compensate for a lesser robustness.

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REFERENCES

1. Advisory Committee on Dangerous Pathogens. Categorisation of pathogens according to hazard and categories of containment. London: HMSO, 1983: 48.
2. Collins CH. Laboratory-acquired infections: history, incidence, causes and prevention, 2nd ed. London: Butterworths, 1988: 295.
3. Jemski JV, Phillips GB. Aerosol challenge of animals. In: Gay WI, ed. Methods of animal experimentation. New York: Academic Press, 1965: 273–341.
4. Newman-Taylor AJ, Longbottom JL, Pepys J. Respiratory allergy to urine proteins of rats and mice. Lancet 1977; ii: 847–9.
5. Druett HA. A mobile form of the Henderson apparatus. J Hyg 1969; **67**: 437–48.
6. Goldberg LJ, Watkins HMS, Boerke EE, Chatigny MA. The use of a rotating drum for the study of aerosols over extended periods of time. Am J Hyg 1958; **68**: 85–93.
7. Walsh M, Pritchard JN, Black A, Moores SR, Morgan A. The development of a system for the exposure of mice to aerosols of plutonium oxide. J. Aerosol Sci 1980; **11**: 467–74.
8. Home Office. Code of practice for the housing and care of animals used in scientific procedures. London: HMSO, 1989: 33.
9. British Standards Institution. Environmental cleanliness in enclosed spaces. BS 5295. BSI: London. 1989.
10. British Patent No. 90/10799.6. Animal housing systems. Br Patent Appl No. 90/10799.6. Applicants Cambridge Isolation Technology Ltd, and University of Bristol. Application filed 14 May 1990.
11. Advisory Committee on Dangerous Pathogens. Guidance on the use, testing and maintenance of laboratory and animal flexible film isolators. London: HMSO: 1985: 12.
12. May KR. The Collison nebulizer: description, performance and application. J Aerosol Sci 1973; **4**: 235–43.
13. Chatigny MA, Clinger DI. Contamination control in aerobiology. In: Dimmick RL, Akers AB, eds. An introduction to experimental aerobiology. New York: John Wiley, 1969: 194–263.
14. Cox CS. The aerobiological pathway of microorganisms. Chichester: John Wiley, 1987: 293.
15. Lane-Petter W. A ventilation barrier to the spread of infection in laboratory animal colonies. Lab Anim 1970; **4**: 125–34.
16. Clough G, Hill A, Blackmore DK. Evaluation of a filter rack for laboratory rodents. Lab Anim 1973; **7**: 149–59.

17. Beall JR, Torning FE, Runkle RS. A laminar flow system for animal maintenance. Lab Anim Sci 1971; **21**: 206–12.
18. McGarrity GJ, Coriell LL. Mass airflow cabinet for control of airborne infection of laboratory rodents. Appl Microbiol 1973; **26**: 167–72.
19. Keller GL, Mattingly SF, Knapke FB. A forced-air individually ventilated caging system for rodents. Lab Anim Sci 1983; **33**: 580–2.
20. Jenkyn JF, Hirst JM, King G. An apparatus for the isolated propagation of foliar pathogens and their hosts. Ann Appl Biol 1973; **73**: 9–13.
21. Clough G. Environmental factors in relation to the comfort and well-being of laboratory rats and mice. In: Standards in laboratory animal management. UFAW, Potters Bar, 1984: 7–24.
22. Yamauchi C, Fujita S, Obara T, Ueda T. Effects of room temperature on reproduction, body and organ weights, food and water intakes and haematology in mice. Expl Anim 1983; **32**: 1–11.