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The safety of the Trexler isolator as judged by some physical and biological criteria: a report of experimental work at two centres

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SUMMARY

We have assessed the effectiveness of flexible-film negative-pressure isolators by physical and biological means. We have found that they afford a high degree of containment and therefore also of safety to hospital staff. We offer some recommendations on the operation of these isolators to ensure the optimum degree of protection.

INTRODUCTION

Flexible-film positive-pressure isolators have been shown to be effective in maintaining microbial isolation of laboratory animals (Trexler, 1971) and have been used for the protection of immune-impaired patients (Trexler, Spiers & Gaya, 1975). Negative-pressure isolators of similar pattern have been developed to protect staff against dangerous infections without greatly interfering with medical and nursing care of the patient (Emond, 1976; Trexler, Emond & Evans, 1977).

A small negative pressure is maintained within the isolator to ensure that there is no escape of contaminated air to the surroundings. Air for ventilation, upon entering and leaving the isolating envelope, passes through high efficiency filters made of glass fibre filter paper media with a penetration of less than 0·003 % against a sodium cloud (particle size ranging from 0·2–2 μm) in accordance with B.S.S. 3928. These filters are sealed at the factory in stainless steel housings and are tested to assure the absence of leakage at the filter mounting. Flexible plastic sleeves, forming a portion of the isolating envelope, fit tightly over cylindrical projections on the filter-housings to provide secure seals when taped in place.

The attendant staff are physically separated from the patient by a barrier of polyvinylchloride film forming the envelopes and by rubber gloves attached to half-suits and sleeves welded to the side-walls of the envelopes (see Fig. 1). All supplies are taken into the isolator through an entry port in the supply envelope

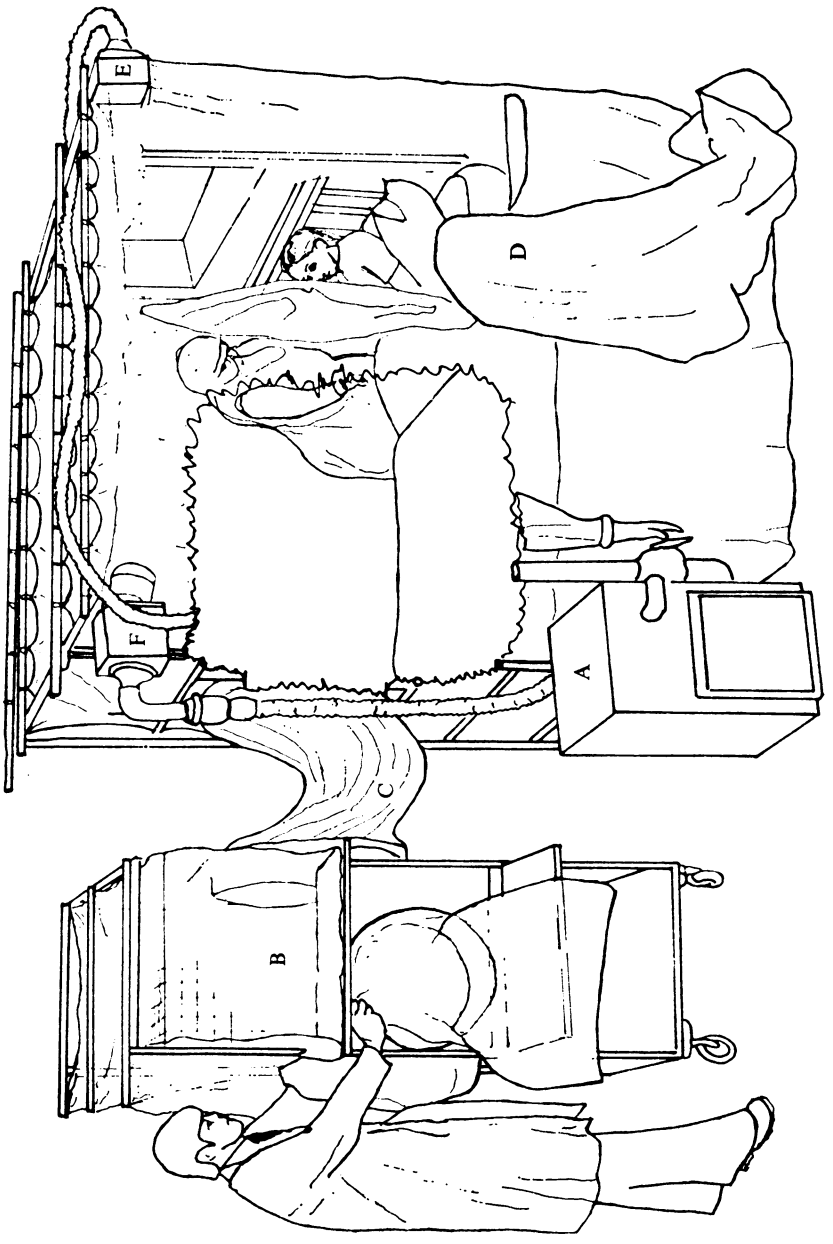

Fig. 1. Containment bed isolator showing a patient in bed, a nurse in a half-suit, and the entry port on the supply trolley in use. A = Air supply unit. B = Supply trolley. C = Attachment sleeve. D = Half-suit. E = Supply airfilter. F = Exhaust airfilter.

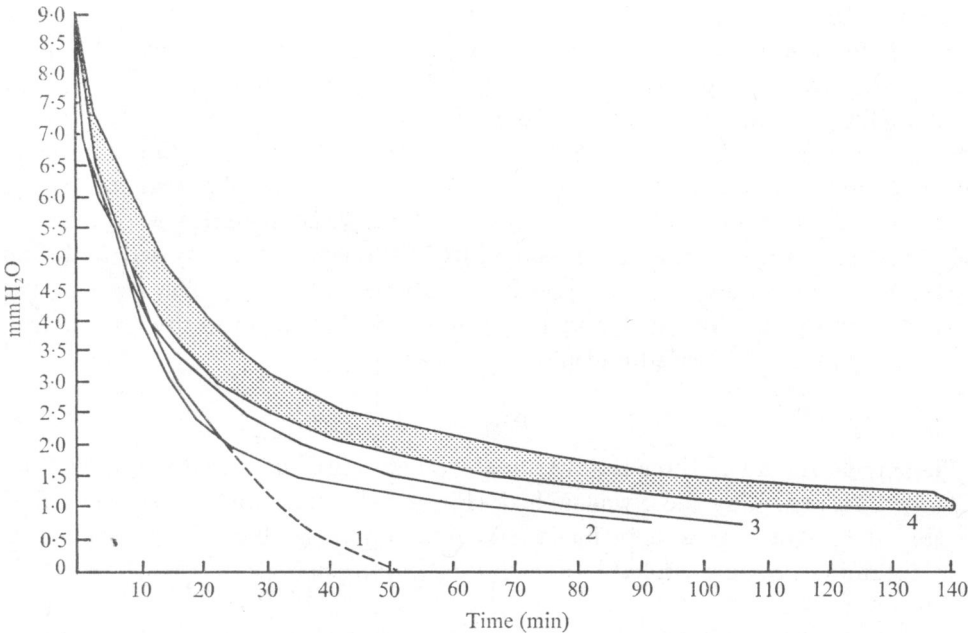

Fig. 2. Pressure tests of isolator envelopes. 1, Laminated envelope (developed leak while being tested); 2, laminated envelope; 3, laminated envelope; 4, non-laminated envelope (range of six tests).

and all waste material is removed by the same route for disposal by incineration or sterilization. The seal on the entry port is maintained by a tightly fitting polythene bag held in place by a stout rubber band.

EXPERIMENTAL METHODS AND FINDINGS

Two distinct methods of assessing safety were used at two centres. At Coppetts Wood Hospital (CWH) air pressure measurements were made: at East Birmingham Hospital (EBH) experiments were conducted with bacteriophage containment. The details of these are presented in separate and complementary sections A and B, together with a further section C dealing with serological tests.

A. Pressure Testing Experiments (CWH)

Three isolator envelopes were tested. Two were of standard pattern and in general use, one was experimental and manufactured from laminated plastic film. The envelopes were tested under positive pressure because this is more sensitive than negative pressure for detecting leaks or porosity in the plastic film.

The isolators were carefully inspected for visible damage before they were pressurized. The envelopes were inflated by running the air pump with the outlet valve closed. The inlet valve was then shut and the pump switched off. Small tears and punctures were detected by feeling for a draught or listening for the hiss of escaping air. When the site of leakage could not be discovered by these means Freon was released within the isolator and a halogen detector used to locate

the defect. Once all leaks had been sealed the envelope was inflated to a pressure of 22 mm H_2O in order to stretch the plastic film and thereby reduce any subsequent drop in pressure as a result of further stretching under test conditions.

An inclined liquid-manometer calibrated in mm H_2O was placed outside the isolator and connected to the interior by a tube passed through a finger in one of the rubber gloves and taped in position to prevent leakage. The manometer was standardized to atmospheric pressure before use and all readings were taken at the same temperature. The air pressure within the isolator was raised to 16 mm H_2O above atmospheric pressure and both valves were closed. The pressure was measured at regular intervals and the time taken for the pressure to fall from 9 mm to 1 mm was recorded and plotted.

Results

The envelopes of the three isolators maintained a pressure greater than 0·2 mm H_2O for 18 h providing there was no leak (Fig. 2): the pressure fell to zero if a leak developed. Contrary to expectations the laminated plastic film proved less effective in maintaining pressure probably due to its greater elasticity.

B. BACTERIOPHAGE EXPERIMENTS (EBH)

T3 phage is slightly smaller (57 nm) than the Arenavirus of Lassa fever (80–100 nm) and of similar or possibly greater stability. On Oxoid D.S.T. agar, inoculated with susceptible *Escherichia coli* (detector plates), this phage produces large easily seen plaques. It was used to detect escape of virus from the isolator into the surrounding air and also the decay rate of virus within the isolator when the pressures were negative, positive and equal to atmospheric. Using a nasal spray 2 ml of T3 coliphage suspension, containing 10^{10} plaque-forming units (p.f.u.) per ml, were released into the supply section of a standard isolator. Detector plates were placed at sites inside and outside the isolator and serially exposed by removal of the plate lid, usually for five minutes during the course of an hour. After incubation of the detector plates at 37 °C overnight the number of plaques or percentage lysis was noted.

These tests were validated by other experiments which showed that at the end of about 60 min when phage numbers in the air were low, visible phage could be recovered in large numbers by swabbing from the inside surface of the plastic envelope: 24 h later phage could be recovered in only small numbers, and at 48 h no phage was detected, either from these sites or by exposing settle plates with concomitant thumping of the envelope. The phage was therefore considered to be stable enough for us to draw the conclusions that follow but not so persistent as to render them invalid. We have previously used this phage as a useful indicator of viral spread (Report, 1975).

Table 1. *Typical results from eight experiments with the isolator adjusted to have a negative pressure as for normal operation*

(Approximate percentage lysis of bacterial lawn or number of phage plaques (p) is given. Site 1. Patient area inside isolator. Site 2. Interior of service isolator. Site 3. Area outside entry port of service isolator. Site 4. Exhaust outlet outside isolator. Control plates were left unopened.)

	Time (mins) from end of spraying					
Site	0–5	15–20	30–35	45–50	60–65	Controls
1	100%	90%	50p	5p	0	0
2	100%	90%	22p	2p	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0

Group 1. *Experiments with negative pressure in isolator*

The controls of the isolator were set to provide minimum negative pressure with an air intake of 950 l/min and 2 ml of T3 coliphage were sprayed into the supply isolator. Settle plates were arranged on the bed in the patient's section, in the interior of the supply section, in the area adjacent to the exterior of the supply port and also in the direct flow of air from the exhaust pipe; covered control plates were also included. A total of eight experiments was conducted at EBH.

Typical results are shown in Table 1. The settle plates exposed for five minute periods showed that numbers of phage were initially uncountable but declined to zero (or one 'break through' plaque) by 50–60 min. It is important to note that bacteriophage was not detected outside the isolator adjacent to the entry port nor in the exhaust airflow by settle plate *at any time* in the absence of manipulations of the sort mentioned below: over 100 settle plates were exposed.

Simulation of faulty techniques and accidents

(*a*) The disposal bag was ligated without preliminary disinfection and cut with an electrically heated cautery. No phage was detected in the air on eight occasions. However, phage was present on the cut surfaces in half the tests, and examination of the bag under water showed leakage of air from a small area of the cut surface. When the inside of the bag was swabbed with Cidex (2½ % glutaraldehyde) before ligation and separation, no phage was detected on the cut surface.

(*b*) On two occasions, when there was a high phage count within the isolator, the disposal bag was deliberately pulled off to simulate an accident. No phage was detected in the area immediately outside the isolator by settle plates.

(*c*) An 8 cm cut was made with a knife in the side-wall of the supply isolator envelope near the entry port. Detector plates were placed outside the isolator over the slit during periods when phage concentration was high inside. The side-wall of the supply isolator was then heavily thumped with the flat of the hand 4 to 13 times. On 6 occasions no phage was detected but on 1 occasion 8 p.f.u. were

Table 2. *Result of one experiment at atmospheric pressure with isolator pump switched off*

(Sites of exposed plates are the same as in Table 1. Detector plates at sites 3 and 4 were left exposed for 60 min for maximum sensitivity.)

	Time (mins) from end of spraying					
Site	0–5 (%)	15–20 (%)	30–35 (%)	45–50 (%)	60–65 (%)	Controls
1	100	90	90	85	80	0
2	100	90	90	85	80	0
3	0		Plates open for 1 h		0	0
4	0		Plates open for 1 h		0	0

present (this was in an early experiment when the degree of negative pressure was not specifically noted).

(*d*) Swabs taken from the inner surfaces of apparently intact rubber gloves of the half-suits and from gloves on sleeves at other sites after use were all found to be phage free. However, as was expected, if a glove was lacerated the inside of the glove and the operator's finger or hand became contaminated with phage – no hand disinfection was used during these tests. Bacteriophage was not detected in the air outside the isolator during replacement of a torn glove even though the fingers of the glove were contaminated.

Group 2. Experiments with atmospheric pressure in isolator

The isolator was left with the ventilation pumps switched off, in order to assess the natural decay rate of aerosol phage at zero air exchange and at atmospheric pressure. Phage suspension (2 ml of 10^{10} p.f.u./ml) was sprayed into the supply isolator, detector plates being exposed in the usual way (Table 2). In two experiments the phage concentration at 60 min was still high, with about 80 % lysis on the detector plates. The adhesive tape sealing the hole in the envelope of the supply isolator was removed and the side-wall thumped. Detector plates showed more than 50 plaques immediately after spraying, but only 6 plaques after 30 min when the number of phage particles in the isolator air was smaller. Six control detector plates, left open for 1 h instead of 5 min at the entry port and exhaust vent sites, showed 1 and 4 p.f.u. on 2 plates; these airborne particles presumably escaped through the hole in the envelope during thumping. In the second experiment of this Group when the hole was left sealed the control plates outside remained phage free.

Group 3. Experiment with positive pressure in isolator

On one occasion the air flow was adjusted to provide a positive pressure within the isolator and an input 950 l/min. The phage suspension was atomized as in the above experiments at the same concentration. The phage decay rate within the tent was slower in this positive phase than in the negative one, probably due to a lower exchange of air. During the experiments the pattern of phage decay was very similar to that of the Group 2 experiments shown in Table 2. When the

disposal bag was deliberately pulled off in this experiment phage was immediately detected outside the tent in large numbers: 90 % lysis at a site adjacent to the bag and 50 % lysis four feet away.

Group 4. Experiments on persistence of phage

Tests were made to see how long phage would persist inside the isolator on the plastic film after completing the experiments. The following results were obtained:

(*a*) At the end of two experiments wet swabs were taken from an area of the plastic envelope, approximately 10 cm square, inside the patient isolator and also inside the supply isolator – a large amount of phage was present (100 % lysis) on all samples.

(*b*) 24 h later swabbing was repeated. Phage was recovered in small numbers (13 p.f.u.) from the supply isolator in one of two experiments only; phage was not found in the swab from the patient isolator. Settle plates were also exposed in the patient and supply isolator and the side-walls of the envelope were thumped violently – no phage was detected.

(*c*) After 48 h further swabs and settle plates were examined and no phage was evident in the two experiments. It was thus concluded that the results were not likely to be influenced by carry over of phage from one experiment to the next after an interval of 24 h.

C. Serological Tests on Hospital Staff

Further evidence on the safety of the isolator was obtained at Coppetts Wood (CWH) where we admitted a patient with Ebola virus infection (Emond *et al.* 1977) and had an opportunity to study the spread of infection to medical and nursing staff. The patient was nursed in a standard isolator throughout the acute stage of his illness, and remained there until clearance tests proved negative, a total of 32 days. Serum samples were taken after a minimum of six weeks from 28 of 29 staff involved and were tested by a fluorescent antibody technique for evidence of Ebola virus infection. No antibody to this virus was detected in any of the sera.

DISCUSSION

The tests at CWH reported in Section A demonstrated that with the air pump switched off and the valves closed a pressure gradient was maintained for many hours between the interior of the isolator and the surrounding room. In the negative-phase this pressure difference would afford protection in the event of pump failure, certainly for sufficiently long to change pump units. However, it would be inadvisable to risk creating a positive pressure within the isolator as a result of displacing air by wearing a half-suit or moving the side-walls of the envelopes before the air pump had been replaced. Theoretical calculations indicate that there would be no appreciable change in oxygen or carbon dioxide levels within the isolator for several hours and therefore no need to remove the patient from the isolator because of respiratory difficulties.

To ensure maximum protection the envelopes of the isolator should be carefully

inspected for visible damage before use. Particular attention should be paid to rubber gloves, to the attachment of glove rings to the sleeves, to the sleeves connecting the patient's envelope to the filters and to entry points for cables and tubes. It is helpful to keep a register of all leaks found during testing and actual use of the isolator to identify points where leaks are likely to occur. Ideally an isolator should be pressure-tested before use to ensure freedom from leaks and maximum security.

In experiments B the isolators were subjected to the very exacting tests of introducing a large number of phage particles into the internal atmosphere, probably exceeding by at least a thousand times the number of virus particles likely to be liberated by any patient. The phage used is smaller than either Lassa or Ebola virus. Although comparable figures for the stability of these viruses are not available it might be expected, on the basis of knowledge of viruses in these groups, to be more stable than either; T3 phage is a double-stranded DNA virus, resistant to lipid solvents, whereas the others are single-stranded RNA viruses sensitive to lipid solvents. These investigations confirmed that the exhaust HEPA filter is safe for particles the size of viruses. They also established that the isolator gives a high degree of protection against leakage from accidental puncture or inadvertant removal of the bag sealing the entry port, providing a negative pressure is maintained. Experimental contamination by virus was greater and the deliberate thumping of the envelope sides was probably a more severe test than would be expected under normal conditions of use.

Detection of phage at the cut ends of the untreated disposal bag emphasizes the importance of preliminary swabbing with disinfectant before ligation. In addition it should be noted that the cautery does not leave an air-tight seal and that squeezing of the bag or the residual stump should be avoided. As would be expected the attendant's hand would inevitably be contaminated if the glove tore and this contamination would be transferred to the half-suit on withdrawing the hand. If a tear was discovered it would be prudent to apply disinfectant to the glove from inside the isolator. Greater protection would be obtained from wearing an additional pair of disposable gloves.

It is important to circulate the air from the isolator continuously through the extract filter to remove as many virus particles as possible and thereby reduce the pool of infection within the isolator. A negative pressure must be maintained within the isolator to prevent leakage. The pressure gradient between the interior of the isolator and the room is very small and not easily measured by a water manometer. However it is quite easy to assess the pressure difference by observing the indrawing of the side-walls of the envelopes. Once the pressure controls have been correctly adjusted it is important that inexperienced staff should not tamper with them and run the risk of abolishing the pressure gradient. An alarm system is provided to draw attention to a rise in pressure from a pre-set value.

Our experiments indicate that the isolator, when used correctly, offers a very great measure of staff protection. This opinion is supported by practical experience at CWH with Ebola virus infection when staff were potentially exposed to the virus for a prolonged period yet did not develop antibody to the infection. Bacterio-

phage is a very sensitive indicator because a single virus particle will generate visible bacterial effects. We have, on the other hand, no idea how many particles of Lassa or Ebola virus are necessary to initiate disease in man though it is likely that man is more resistant to infection by these agents than *Escherichia coli* is to T3 phage. Our experiments probably err on the side of safety for the amount of bacteriophage liberated in the isolator exceeded by several orders of magnitude the titres of virus found in the nasopharynx in such highly infectious diseases as influenza. Although the isolator has been shown to be efficient we would regard it as essential that it should be sited within a high security unit with full supportive services so that in the event of an accident of the type deliberately created in our experiments or the presence of a violent patient there is a second line of containment.

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