



Advisory Committee on Dangerous Pathogens

GUIDANCE ON THE USE, TESTING AND MAINTENANCE OF LABORATORY AND ANIMAL ISOLATORS FOR THE CONTAINMENT OF BIOLOGICAL AGENTS – Second Draft

Issue

1. Consideration of the second draft of the guidance document and an update on comments received during the consultation process.

Background

2. HSE is updating and replacing the 1985 ACDP publication: '*Guidance on the Use, Testing and Maintenance of Laboratory and Animal Flexible Film Isolators*'. This will be a web-based document with the ability to update and add additional sections when they become available.
3. The second (most current) draft is included at Annex A.
4. This version was circulated to the wider biological sciences community in spring 2006 and comments summarised below.

Summary of comments from consultation

5. The main areas where consultees felt further work is required are listed below:

Section 4 - types of isolators

6. It was felt that the guidance would benefit from a more detailed descriptions of the different types, expanding on the pros and cons and being clear about when each type should be selected and used.

Section 5 – commissioning tests

7. It was felt that listing specific figures (e.g. overpressure of 150 Pa, numbers of air changes etc) could be confusing for those centres where these could not be met and/or may result in cursory risk

assessments for those centres where they could. As such there was a preference for emphasising the principles of risk assessment in deciding the importance of the different measures, rather than specifying actual figures. It was also felt that 'vague language' should be avoided to improve clarity.

Section 6 – maintenance testing

8. It was felt that more work was needed here to improve the presentation, be clearer about the intervals of testing and ensure compatibility with other guidance.

Action

9. Members are invited to comment on the second draft and the comments received during the consultation.
10. Members are asked to decide whether they wish to see a copy of the next draft or whether they are happy for HSE to work up the comments and submit directly for publication later in 2006.

Secretariat

September 2006

GUIDANCE ON THE USE, TESTING AND MAINTENANCE OF LABORATORY AND ANIMAL ISOLATORS FOR THE CONTAINMENT OF BIOLOGICAL AGENTS

1. BACKGROUND

1. This guidance is issued by the Health and Safety Executive (HSE). Following the guidance is not compulsory and you are free to take other action. But if you do follow the guidance you will normally be doing enough to comply with the law. Health and safety inspectors seek to secure compliance with the law and may refer to this guidance as illustrating good practice.

2. INTRODUCTION

2. This guidance gives advice on factors to consider when selecting, using, testing and maintaining negative pressure isolators for experiments involving biological agents^a and replaces previous guidance issued in 1985¹. The main scenarios covered by this guidance are laboratory isolators for housing equipment/large experiments etc and animal husbandry isolators for animals infected with biological agents. Pharmaceutical isolators, particularly those operated at positive pressures are covered by separate publications².
3. It is aimed at employers, laboratory managers, safety advisors and users. Other groups such as manufacturers and companies which offer testing and maintenance services may also find it useful.
4. It applies in situations where isolators are selected as the mechanism of primary containment, usually because the task is unsuitable to carry out in a microbiological safety cabinet. It does not cover other laboratory or animal containment strategies such as individually ventilated cages (IVCs) or downdraft tables. The main reason for this is current limited knowledge on the effectiveness of these alternatives for adequately containing aerosols of biological agents, and the range and complexity of testing required to validate this containment. This is not to say that these alternatives cannot be used for work with biological agents, but that it will fall to dutyholders to undertake the relevant testing and monitoring to demonstrate ongoing containment. Given that the use of IVCs is expanding in this area, it is likely that more data will become available to hopefully enable supplementary guidance to be made available in the future.

^a A biological agent is defined in COSHH as: ‘*a microorganism, cell culture or human endoparasite, whether or not genetically modified, which may cause infection, allergy, toxicity or otherwise create a hazard to human health*’.

3. LEGAL REQUIREMENTS

Health & Safety Issues

Biological Agents

5. The Control of Substances Hazardous to Health Regulations 2002 (as amended)³ (COSHH) require employers to prevent, or if this is not reasonably practicable, adequately control exposure of employees to biological agents using measures other than personal protective equipment. Where exposure cannot be prevented employers must take steps to ensure that exposure is adequately controlled by using a combination of control measures specified in Schedule 3 of which states that *infected material, including any animal is to be handled in a safety cabinet or isolator or other suitable containment.*
6. The Genetically Modified Organisms (Contained Use) Regulations 2000⁴ (as amended) require suitable containment and control measures be used to adequately control against exposure to biological agents which are genetically modified and the Genetically Modified Organisms (Contained Use) (Amendment) Regulations 2005 specifically state that work with infected animals at containment levels 3 and 4 must be undertaken in isolators.
7. For small scale *in vitro* laboratory activities a microbiological safety cabinet (MSC) is widely recognised as offering a practicable engineering solution for controlling aerosols. However for non-standard laboratory activities (such as those involving large pieces of equipment) or animals infected with biological agents, it may not be practicable to use an MSC.
8. Isolators that are used, tested and maintained appropriately are recognised as being able to provide effective engineering control against aerosols from biological agents associated with such activities. However, fundamental to the whole process is a robust risk assessment addressing the specifics of the activities to be undertaken and taking into account relevant guidance, for example working safely with research animals⁵.

Other Health & Safety issues

9. Whilst the primary use of isolators is to control exposure of operators to aerosols of biological agents, it is important to take into account other health and safety issues, including human factors such as ergonomic considerations, in the risk assessments. Further information on this subject can be found in Annex A.

Animal Welfare Issues

10. Animal welfare requirements are specified by the Animals (Scientific Procedures) Act 1986. The associated Home Office Code of Practice⁶ specifies, amongst other things, the minimum environmental parameters that must be met in relation to space, noise, temperature and humidity etc for different animal species.

Environmental Issues

11. The GMO Contained Use Regulations require that appropriate measures be in place to protect the environment as well as human health. In addition, the Specified Animal Pathogen Order 1998⁷ requires the containment of exotic animal pathogens so that the environment is protected. Isolators may be a useful form of primary containment to reduce the likelihood of an environmental pathogen escaping.

4. TYPES OF ISOLATORS

12. During selection of an isolator consideration of the main task(s) to be carried out along with related activities (for example, the movement of samples, removal of waste etc) is essential. Further information regarding isolator selection can be found in Annex B.

Flexible Film Isolators

13. Flexible Film Isolators (FFIs) are tented enclosures of flexible plastic built on a metal frame. They are routinely accessed from the side through gauntlets or sleeves with cuffed gloves. Ports allow material to both enter and exit the isolator.
14. The size of these units and therefore the amount of equipment within is limited by the degree of access afforded by the gauntlets.

[DN – a picture will be inserted here – to be sourced]

Rigid Isolators

15. These are essentially the same as FFI's except their canopies are made of a rigid material.

[DN – a picture will be inserted here– to be sourced]

Flexible half-suit isolators

16. These are larger than FFIs and are accessed by the operator by half suit(s) in the base of the isolator and also by gauntlets/sleeves and gloves on the side. Operators enter the half suit by ducking under the base and then standing up within the positively pressurised suit.
17. This combination gives the potential for large work surfaces within the isolator due to the increase in accessibility.

[DN – a picture will be inserted here– to be sourced]

Rigid half-suit isolators

18. These are essentially the same as flexible half suits isolators except their canopies are made of a rigid material. This makes them more resistant to damage, but less able to absorb pressure fluctuations, for example when entering or exiting the half suits.

[DN – a picture will be inserted here– to be sourced]

Transfer isolators

19. These are FFIs or rigid isolators used specifically for transferring materials from one isolator/MSC to another. They are usually battery powered and have docking ports to enable attachment and transfer.

5. COMMISSIONING TESTS FOR ISOLATORS

20. Whilst MSCs have a defined British Standard⁸, isolators currently have no such equivalent (although general guidance is given in Annex A of the Laboratory Operations Standard⁹). From a containment perspective there are many similarities between an isolator and a Class III MSCs and wherever possible, isolators should aim to provide a similar level of protection as afforded by the MSC. Following the testing and maintenance regimes in this document will usually ensure that the expected requirements are met.
21. Commissioning of an isolator should be when it is in its final position within the laboratory/animal room. Factory commissioning only is not acceptable. Commissioning will also be required after any major changes to the isolator such as following dismantling and/or movement and additions/alterations.
22. The following 5 tests are considered essential at commissioning:
- air leaktightness
 - leak detection
 - filters
 - negative pressure
 - air change rates

Air Leaktightness

23. The isolator should be leak-tight in order to control exposure to aerosols of biological agents and also gaseous agents (e.g. formaldehyde) used in the disinfection or task process. The MSC British Standard requires a positive pressure hold test be undertaken to determine the air leaktightness of a Class III MSC. The specified overpressure of 500 Pa is unlikely to be practicable in an isolator, particularly film isolators. Instead a practicable alternative is considered to be a minimum overpressure of 150 Pa, as determined by the supplier.

24. A suitable pass criteria would be no more than a 10% loss of pressure when the internal isolator pressure is raised to 150Pa and held for 30 minutes. One way of assessing this is described in the information box.

Information Box

One method for undertaking positive pressure hold test: the isolator should be assembled with all connections (gloves, gauntlets, half suits etc) made. The extract air duct should be blanked off and the internal pressure raised to 150 Pa by the supply air, which should then be blanked off. For flexible isolators the canopy should be allowed to stretch fully (e.g. for 30-60 minutes) before the 150 Pa pressure is re-established. The supply air should be finally blanked off and the pressure noted at 5 minute intervals over a 30 minute period. Over this time the pressure of 150 Pa should be maintained within a 10% margin.

Leak detection

25. Leak detection should still be undertaken whether or not the isolator passes its pressure hold test. This is because the hold test relates to leak rates, which allows for a certain amount of breach in the containment before the test fails. Leak detection is important in order to identify minor breaches, which could deteriorate during use.
26. The particle tracer test is the recommended method for leak testing given that it is sensitive, relatively easy to perform and uses the same type of equipment as for filter testing. In addition it can be undertaken at the same time as the positive pressure hold test. One way of assessing this is described in the information box.

Information Box

One method for undertaking particle tracer test: place a hot or cold smoke generator capable of generating aerosols of small (e.g. 0.3 micron) particles (e.g. food oil Ondina DL) within the isolator. Raise the internal pressure to minimum of 150 Pa and blank off the supply and extract air. Use a photometer to scan the whole canopy, filter housings etc.

Filters

27. H14 HEPA filters, meeting the relevant standard¹⁰, must be in place for the extract air; double HEPA filters must be used if the exhaust air is to be re-circulated into the laboratory and the supply and extract fans must be interlocked to prevent positive pressurisation. In addition, filtration may also be required for supply air, if animal protection is needed.

28. The filters must be seated appropriately and all must be fitted with access ports to allow independent, full face scan testing. The test should meet the standard listed above, with particulate concentrations on both sides of the individual filters measurable.

Negative pressure

29. There will be a broad range of acceptable working pressures, influenced by airflow rates, type of isolator and activity. However, a minimum pressure of 30 Pa below the air pressure of the laboratory should be maintained.
30. As a minimum, positive pressurisation must not occur during normal working practices, which includes when operators enter and leave the isolator (e.g. through half-suits). In addition, the pressure differential should be such that negative pressure is maintained in the event of a foreseeable breach in the isolator envelope.

Air change rates

31. As for negative pressures, the air change rate selected will depend on the type of isolator and the activity. The rapid removal of air potentially contaminated with aerosols reduces the likelihood of accidental exposure and the higher the air change rate the more rapidly aerosols can be removed. In addition, the higher the rate, the lower the waiting time required for disinfection between tasks and following any spills within the isolator.
32. High levels of air change rates are considered inappropriate when housing experimental animals. The Code of Practice for the Animals (Scientific Procedures) Act requires that animals are not kept in draughts with controlled ventilation, noise, humidity and temperature. For example, it is recommended that rodents should have 15-20 changes of fresh air per hour. It is however, generally considered that animal husbandry with infected animals is less likely to generate aerosols of biological agents of high concentration and as such lower levels of air change rates may be appropriate. A minimum of 13 air changes per hour (ach) should be in place.
33. Activities involving laboratory work and pieces of equipment are likely to have the potential to generate aerosols of high concentration and as such high levels (e.g. 40 ach) may be required. In addition, equipment housed within isolators are likely to generate heat hence higher air change rates will assist in dispersing this and controlling heat stress to the canopy.
34. One way of assessing air change rates is described in the information box.

Information Box

One method for calculating air change rate: measure the mean airflow velocity at the exhaust with an anemometer, multiply this by 3600 and divide by the isolator volume.

35. Summary of requirements of tests required at commissioning of isolator

Requirement	Value
Air leaktightness	No more than 10% loss of pressure when held at +150 Pa for 30 mins
Leak detection	Pressure held at +150Pa with no leaks detected
HEPA filters	Following BS EN 1822:2000
Negative pressure	At least –30Pa below laboratory pressure
Air change rate	Minimum of 13 air changes per hour

6. MAINTENANCE TESTING OF ISOLATORS

36. Isolators are considered as local exhaust ventilation systems under COSHH and as such are required to have regular examination and testing at intervals of not more than 14 months. At containment level 3 and 4 it is recommended that this be done every 6 months, in line with the recommendations for MSCs¹¹.
37. If it is not practicable to test regularly, for example because an animal study runs for longer than 6 months, then robust 'in use' testing should be carried out and documented to ensure the isolator continues to provide an adequate control system. In addition, because isolators tend to be more fragile than MSC's and more prone to damage during normal operation regular in use testing is essential.
38. The following is a non-exhaustive list of maintenance tests along with their recommended frequency.

	Test	Minimum frequency	Reasons
Calibration of manometer(s).	Calibration	annually	for pressure and air flow rate
Monitoring of manometers	Visual	on use	for pressure and air flow rate.
Isolator surfaces, including the extract ducting.	Visual	6 monthly	examined for defects, cracks and any other damage.
Inspection of the canopy, gauntlets/sleeves and gloves and/or half suit.	Visual	on use	Any damage should be dealt with immediately
Integrity of canopy tested	Soft soap, smoke pencil, careful visualisation (see information box)	6 monthly	Repair breaches in the canopy for example, using silicone sealant and/or patching
Integrity of gauntlet/gloves tested	Soft soap, smoke pencil, careful visualisation	6 monthly	Replace and test new before starting next study
Alarm testing		6 monthly	Confirm functioning correctly
Anti blowback valve		6 monthly	Confirm functioning correctly.
Integrity of filter and housing confirmed.	Full face scanning	6 monthly	replace as required
Inlet filter.	Anemometer readings	6 monthly	can give an indication of filter blockages over time
Housekeeping inside isolator		on use	Should be kept clean and free of unnecessary equipment.

Information Box

One or more of the following tests could be used to detect damage and leaks in the canopy, half suits, gauntlets/gloves.

Soft soap

Raise the internal pressure to minimum of 150 Pa and apply 2.5% solution of soft soap in tepid, distilled water to all welds, gaskets & joints using a paintbrush. Any leaks should be identifiable by the creation of bubbles.

Smoke pencil

Raise the internal pressure to 150 Pa and monitor all welds, gaskets, joints filter housings etc using a smoke pencil. Any leaks should be visualised by deflections to the smoke plume.

Careful visualisation

Use a plate to seal off the glove across the port/shoulder ring. Monitor the pressure change within the glove system. If it equilibrates, rate of change can provide direct assessment of leakage.

Place a flexible diaphragm across a glove port/shoulder ring to trap air within the glove system. If there is leakage the air within the glove will be drawn into isolator (and then out through filter), causing the diaphragm to adopt a visible concave shape.

ANNEX A**Human Factors**

It is important that human factors along with other health and safety issues are taken into account when working with isolators. Human factors can be described as the interaction between the user, task and equipment including behaviours and ergonomics. Selection and use of an isolator needs to be driven by the requirements of the operator and not solely by the task. For example, lack of consideration to good ergonomic practice can result in reduced work efficiency, user discomfort, injury and compromised safety.

For example, animal husbandry is a physically demanding occupation involving a great deal of lifting and carrying with the majority of the work time spent standing. Isolators used for animal work need to be selected to meet the requirements of the animals and to achieve the best ergonomic performance for the user.

An assessment of the manual handling risks should be undertaken referring to relevant guidance¹² to avoid unacceptable strain due to the type of work and/or amount of equipment. It may be necessary to select a different type of isolator or adapt/reduce the cages/equipment designated for the study.

Ergonomic issues to consider before selecting an isolator:

- The physical and psychological characteristics of the users.
- Training and supervision.
- The procedures to be undertaken i.e. whether manual, gross or fine finger dexterity is required.
- The reduction of manual dexterity when working in an isolator.
- Access and egress, especially with half suit isolators where the user has to duck down under the equipment to enter the suit.
- Positioning of equipment inside the isolator so that they are within a zone of convenient reach.
- The best ergonomic performance for example, half suit isolators provide the user with the ability to twist and rotate, whereas FFI's do not.
- The frequency of breaks or changes in task to reduce fatigue.
- Means of communication e.g. if working in half-suit isolators

Before final selection it may be advantageous to put together a 'mock up' to ensure all the ports, gloves, half suits etc are situated in the best positions for the task to be carried out and to provide hands-on training of staff.

The nature of research, diagnostic and animal work means that it may be necessary to work out of hours and/or alone. A local risk assessment will identify any specific hazards for lone working and the use of isolators in the containment laboratory.

ANNEX B**Factors to consider when selecting an isolator to ensure that it is fit for purpose**

Canopy	Sealable without the use of excess sealant; able to fumigate
Flexible canopy	Flexible and resistant to tear and disinfectant that will be used during procedures and cleaning
Floor	Hard, resilient surface
Half suit	Connected to port using double rubber seals (not silicone sealant)
Services	Supplied through a metal plate (using gas tight sockets) to prevent damage to canopy – electricity required for fumigation
Filters	H14 HEPA filters on extract; may be needed on supply for animal protection.
Battery back up	Necessary if housing animals, useful for long-term studies.
Location of alarms (e.g. negative pressure)	Good visibility and access
Fans	Supply (if present) and extract interlocked to prevent positive pressurisation.
Pass box	Separate fan and filters (usually with higher air change rate than main isolator) to assist in passing out samples and fumigating out waste. Usually only on larger half suit isolators.
Communication	In half suit isolators may require e.g. headsets to allow communication between users
Waste port	If waste removal required and no pass box present
Docking port	Double ended for attaching transfer isolator or another isolator/MS
Dunk tank	Could be used for sample movement
Entry and exit to half suit isolators	Thought given to where the various ports are sited and ease of access will prevent injuries to users and damage to the isolator.

References

-
- ¹ 'Guidance on the Use, Testing and Maintenance of Laboratory and Animal Flexible Film Isolators' Advisory Committee on Dangerous Pathogens (ACDP)
 - ² Pharmaceutical isolators: A guide to their application, design and control 2004 Eds Midcalf B, Mitchell Phillips W, Neiger JS & Coles T J
Pharmaceutical Press ISBN 0 85369 573 3
 - ³ Control of Substances hazardous to Health Regulations 2002 Approved Code of Practice L5 HSE Books 2005 ISBN 0 7176 2981 3
 - ⁴ A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000 L29 HSE Books 2000 ISBN 0 7176 1758 0
 - ⁵ Working Safely with research animals: management of infection risks (1997)
HSE Books 1997 ISBN 0 7176 1377 1
 - ⁶ Code of practice for the housing and care of animals used in scientific procedures http://www.homeoffice.gov.uk/docs/cop_hcasp.html
 - ⁷ Specified Animal Pathogens Order 1998, SI 1998/463 The Stationery Office (1998), ISBN 0 11 065801 9
 - ⁸ Biotechnology. Performance criteria for microbiological safety cabinets BS EN 12469:2000
 - ⁹ Biotechnology. Laboratories for research, development and analysis. Guidance for biotechnology laboratory operations BS EN 12741:1999
 - ¹⁰ High efficiency air filters (HEPA and ULP) classification, performance, testing and marketing. BS EN 1822:2000
 - ¹¹ The management, design and operation of microbiological containment laboratories Guidance, HSE Books 2001, ISBN 0 7176 2034 4
 - ¹² Manual Handling. The Manual Handling Operations Regulations 1992. Guidance on Regulations L23 HSE Books 1998 ISBN 0 7176 2415 3