PHARMACEUTICAL INSPECTION CONVENTION
PHARMACEUTICAL INSPECTION CO-OPERATION SCHEME

PI 014-3
25 September 2007

RECOMMENDATION

ISOLATORS USED FOR ASEPTIC PROCESSING AND STERILITY TESTING

Editor: PIC/S Secretariat

e-mail: info@picscheme.org
web site: http://www.picscheme.org

TABLE OF CONTENTS

1. DOCUMENT HISTORY

This document: Recommendation on isolators used for aseptic processing and sterility testing, has been derived in part from PS/W 3/97 (Rev. 2) (Inspection of Isolator Technology), which has not been issued, and other sources. The work of the original working group and coordinator is hereby acknowledged.

Adoption by the Committee	24 April 2002
Entry into force	1 July 2002

2. INTRODUCTION

2.1 The term ‘Isolator’ as used in the Pharmaceutical Industry covers a variety of pieces of equipment. One group has the main objective of providing containment for the handling of dangerous materials either aseptically or not. Another group has the main objective of providing a microbiologically controlled environment within which aseptic operations can be carried out.
Containment isolators often employ negative internal air pressure and most isolators used for aseptic processing employ positive pressure. A sporicidal process, usually delivered by gassing, can be used to aid microbiological control. Some large scale isolators provide an opening, often called a mousehole, to permit continuous removal of sealed product. Other isolators remain sealed throughout production operations. The capability for the isolator to be sealed allows operations to be carried out in controlled gaseous environments e.g. anaerobic conditions.

2.2 Aseptic operations can include sterility testing or aseptic processing to produce medicinal products. This Recommendation deals mainly with the provision of a microbiologically controlled environment for aseptic processing for producing medicinal products labelled as sterile. The principles necessary to assure a microbiologically controlled environment for production, are also appropriate, in most cases, for isolators used for sterility testing.

2.3 Controlled environments for aseptic operations are currently mainly provided by conventional clean rooms, of Grade B, containing workstations, of Grade A complying with the PIC/S and EC guide to GMP. A smaller number of controlled environments are provided by clean rooms, of Grade D or better containing equipment called isolators providing a Grade A environment.
When isolators are used for sterility testing there is no formal requirement for them to be placed in a Grade D environment. The environment should be controlled e.g. allow access only to trained staff, but not necessarily classified.

2.4 Isolators could be seen as a more encompassing development of the barriers used in conventional clean rooms. The clean room barriers evolved from plastic flexible curtains through to rigid barriers with glove ports. The objectives of barriers are to increasingly separate the surrounding clean room including the operator from the critical zone where aseptic operations are carried out and sterile materials are exposed. When the degree of containment is nearly complete, the sporicidal procedures used for many years in other applications could be applied without harming the operators.

2.5 Another line of development, more applicable to small-scale aseptic activities, was to shrink the clean room together with its inlet and outlet airlocks to the size of a workstation with airlock hatches. A Grade A internal environment is created and manipulations are carried out through glove ports. The routine application of a sporicidal process to this type of equipment is not carried out, reliance being placed on conventional clean room sanitisation methods. This concept has been developed and implemented to create a type of environmental control equipment that is used for small-scale aseptic operations mainly in hospital pharmacies. These are commonly called isolators by the manufacturers and users.

2.6 This Recommendation addresses only isolators that are subjected to a sporicidal process (usually delivered by gassing) as they are the most frequently found types in licensed industrial facilities.

3. PURPOSE

The purpose of this document is to provide guidance for GMP inspectors to use for training purposes and in preparation for inspections of isolators subjected to a sporicidal process used for aseptic processing and sterility testing.

4. SCOPE

4.1 This Recommendation applies to isolators subjected to a sporicidal process used for aseptic processing and sterility testing.

4.2 At the time of issue, this document reflected current state of the art. It is not intended to be a barrier to technical innovation or the pursuit of excellence.

4.3 The advice in this Recommendation is not mandatory for industry. However, industry should consider these recommendations as appropriate.

5. DEFINITIONS / GLOSSARY

5.1. Pharmaceutical Isolator

An isolator is an arrangement of physical barriers that are integrated to the extent that the isolator can be sealed in order to carry out a routine leak test based on pressure to meet specified limits. Internally it provides a workspace, which is separated from the surrounding environment. Manipulations can be carried out within the space from the outside without compromising its integrity.

5.2. Industrial isolators used for aseptic processing.

Industrial isolators used for aseptic processing are isolators in which the internal space and exposed surfaces are microbiologically controlled. Control is achieved by the use of microbiologically retentive filters, sterilization processes, sporicidal processes (usually by gassing) and prevention of recontamination from the external environment.

5.3. Sporicidal process.

A gaseous, vapour or liquid treatment applied to surfaces, using an agent that is recognised as capable of killing bacterial and fungal spores. The process is normally validated using biological indicators containing bacterial spores. The number of spore log reductions is not specified in this definition, but a target of six log reductions is often applied. The process is applied to internal surfaces of the isolator and external surfaces of materials inside the isolator, when conventional sterilization methods are not required. The application of a sporicidal process to isolators is not considered to be a sterilization process in the same way as, for example, a sealed container subjected to a validated dry heat, moist heat or irradiation process.

5.4. Aseptic techniques and manipulations

The manipulation of sterile materials in such a way as to minimize the risk of microbiological contamination from the environment. These techniques usually involve eliminating surface to surface contacts (except between sterile surfaces) minimizing the area exposed and the duration of exposure.

5.5. Critical zone

Zone within the Aseptic Processing Area where sterile product, product components or product contact surfaces are exposed to the environment.

6. PRINCIPLES RELATED TO THE SELECTION AND USE OF ISOLATORS SUBJECTED TO A SPORICIDAL PROCESS.

6.1 The application of these principles to isolators used for sterility testing may be modified based on a suitable rationale.

6.2 The reasons for selecting an isolator include containment, to protect the operator and environment, and reducing the risk of microbiological and other contamination of the product from the environment.

6.3 This Recommendation focuses on the aspect of reducing the risk of microbiological contamination arising from the environment.

6.4 The general arrangements made to exclude living microorganisms and justify their absence being expected, include the following.

6.4.1 All surfaces that may be contaminated with microorganisms and that are in any way or at any time exposed to the critical zone should be sterilized or subjected to a validated sporicidal process. This includes the resident surfaces of the isolator and transient surfaces of materials moving into and out of the isolator.

6.4.2 None of the surfaces treated above should be exposed to recontamination within the isolator.

6.5 If the isolator is used for aseptic processing the surrounding room should comply with EC Grade D as a minimum. The potential for contamination from the room to enter the isolator during, for example maintenance, should be taken into account when returning the isolator to its production status.

7. DETAILED POINTS TO BE CONSIDERED FOR THE IMPLEMENTATION OF THE PRINCIPLES TO ISOLATORS SUBJECTED TO A SPORICIDAL PROCESS. THESE POINTS ARE EXPANDED UPON IN APPENDIX 1.

7.1 Training of a company team is essential to assure the safe routine operation and requalification of the system.

7.2 The materials that should be sterilized i.e. treated using a validated sterilization process as specified in the Pharmacopoeia, and those that should be subjected to a sporicidal process, should be identified. The rationale used should be documented, the list should be a controlled document and consistent with that used in validation. Particular attention should be paid to microbiologically retentive filters used to treat services to and from the isolator. When the sporicidal process cannot be assured the use of presterilized filters may be necessary.

7.3 The sterilization of equipment, product components and the formulation ready for filling is outside the scope of this document. Their introduction into the isolator system and prevention of recontamination is addressed below.

7.4 Sporicidal process (when non gaseous systems are used the relevant principles should still be applied*)*

7.4.1 The agent selected for gas generation should be sporicidal.

7.4.2 The correct identity and composition of the gassing agent charged into the gas generator should be assured.

7.4.3 Gas generators should not be assumed to be equivalent to each other.

7.4.4 The way in which the gas generator works should be understood by company staff. All critical parameters related to its operation should be identified and recorded throughout the process. Measuring instruments should be calibrated and where critical should have independent monitors or an assured and confirmed reliability. The gas generator should be included in the preventative maintenance program.

7.4.5 The release of the gassing process with regard to the gas generator should verify that all critical parameters met the specifications defined during validation.

7.4.6 The delivery of gas from the generator into the isolator should assure that only the gas generated is supplied. All inlet and outlet filters associated with the isolator should be exposed to gas or sterilized. Any air supplied by the generator e.g. during a purge stage, should be filtered though microbiologically retentive filters that have been sterilized or subjected to a sporicidal process.

7.4.7 The delivery of the correct gas at the validated concentration to the isolator and/or leaving the exhaust system should be confirmed if possible, to supplement the control in 7.4.2

7.4.8 The isolator should be cleaned prior to the sporicidal process. The surfaces of packaged materials and all other items to be gassed within the isolator should be clean.

7.4.9 Delivery of gas or other sporicidal treatment to all necessary surfaces should be assured.

7.4.10 The risk of recontamination of sterilized containers leaving the sterilizing zone and entering the cooling zone of a tunnel integrated with an isolator system, should be evaluated. Consideration should be given to sterilization or sporicidal treatment of the cooling zone.

7.4.11 The sequence of the different aspects of cleaning, sterilization, sporicidal treatment, gassing, and degassing are of critical importance and should be carefully defined and verified before formal release of the system for production.

7.4.12 The range of parameters and events that should be monitored to assure the delivery of the validated process should be defined.

7.4.13 The design, development and validation of the gassing process should encompass all relevant aspects from methods of gas distribution to quantification of target lethality, selection, calibration and culture of the biological indicator and definition of the final protocols. The stage of degassing is critical in all applications and the absence of residual lethality due to inadequate degassing should be demonstrated for isolators used for sterility testing. Reference to Appendix 1 is recommended.

7.4.14 The provisions for requalification and interpretation of results should be carefully and prospectively defined.

7.5 The prevention of recontamination

7.5.1 All gases, fluids and air supplied to the isolator or that may gain access, should be filtered using microbiologically retentive filters or sterilized prior to entry.

7.5.2 The control of leaks between the isolator and surrounding room and between different parts of the isolator system as necessary, should be assured as far as possible. As a guide a minimum of 10 Pascal positive differential air pressure should be maintained to protect against unforeseen circumstances. The maintenance of positive pressure should be monitored and fitted with an alarm.

7.5.3 A program to minimize the risk of loss of integrity of gloves, sleeves and suits should be present. This should include operator practices, vigilance and the absence of sharp edges. There should also be an all encompassing preventative maintenance program that includes specification of examination and preemptive replacements.

7.5.4 Transfer of material out of the isolator should not compromise the critical zone.

7.5.5 Transfer of material into the isolator should not compromise the critical zone.

7.5.6 Air change, laminar/turbulent, aseptic technique, and ergonomics

7.5.6.1 The design of the isolator system should include consideration of air change rate, the use of laminar, unidirectional or turbulent airflow, the application of aseptic technique and risk of error due to human fallibility. The rationale for the decisions taken should be documented.

7.6 Monitoring and testing

7.6.1 Physical monitoring and testing should be based on a systematic failure mode analysis or a suitable alternative and assure the detection of change, failure or aging that could compromise operations.

7.6.2 Microbiological monitoring should take into account the special requirements for sensitivity of testing in isolators subjected to a sporicidal process and avoid compromising operations. The interpretation of results of environmental monitoring should be based on the premise that the detection of any microbiological contamination probably indicates a failure of the system.

7.6.3 Media fills and sterility testing should be carried out as normal for aseptic processing.

8. REVISION HISTORY

Date	Version Number	Reasons for revision
1 July 2004	PI 014-2	➢ Change in the Editor's co-ordinates
25 September 2007	PI 014-3	➢ Change in the Editor's co-ordinates

9. AN EXPANSION OF THE DETAILED POINTS TO BE CONSIDERED FOR THE IMPLEMENTATION OF THE PRINCIPLES TO ISOLATORS SUBJECTED TO A SPORICIDAL PROCESS.

9.1 Principles related to the selection and use of isolators subjected to a sporicidal process.

9.1.1 The principles of design, validation and use should arise from the objectives employed to make the decision to use an isolator subjected to a sporicidal process and the consequent user requirements specifications. It is recognized that the considerations may include operator protection and financial optimization as well as reduced risk of microbiological contamination of the product from the environment. Other aspects that should be considered are the principles of dedication to avoid cross contamination and mix up. These are general principles of GMP, but the particular risk of chemical contamination of the exhaust filters and potential for blowback into the adjacent critical zone should be considered. Standard approaches to design qualification, installation qualification, operational qualification and process qualification are appropriate with the additional provision for periods of development work particularly for the sporicidal process.

9.1.2 This Recommendation focuses on the aspect of reducing the risk of microbiological contamination arising from the environment.

9.1.3 This position leads to an apparent paradox as to whether an isolator that has been subjected to a sporicidal process that demonstrates a classical clean room pattern of the occasional detection of microorganisms, is acceptable for aseptic processing. It is difficult to conceive of an isolator that has been subjected to a sporicidal process being designed and validated to assure and demonstrate clean room performance if it is working correctly. The choice of an isolator that has been subjected to a sporicidal process inevitably drives up the standard; the presence of microorganisms implies that the validated condition probably no longer prevails and the fault should be identified and corrected. Another factor to take into account is the method of use of the isolator. The lower risk of contamination may be used to justify different practices than used in clean rooms; these may include the following:

- Reduced frequency of autoclaving of indirect product contact parts. (See below).
- Extend the allowable period of exposure of sterile surfaces and materials.
- Reduce the need to discard open containers surrounding an intervention via the glove ports.

9.1.4 The above practices would no longer be safe if a return to clean room criteria of performance took place.

9.2 **Training of a company team is essential to assure the safe routine operation and requalification of the system.** An isolator system is still relatively new technology and there is no general knowledge available to the extent that it is available for conventional clean rooms. For this reason it is imperative that the company makes special provisions for training engineers, production and quality staff in the technology including the ergonomic aspects. Abdicating the job of design, installation, development, validation protocols and execution of the protocols entirely to suppliers and consultants is missing a valuable opportunity. The suppliers and consultants should be shadowed by the company team to enable enough knowledge to be accumulated to assure the safe routine operation and requalification of the system. This knowledge should then be cascaded to all people routinely involved.

9.3 **The materials that should be sterilized i.e. treated using a validated sterilization process as specified in the Pharmacopoeia, and those that should be subjected to a sporicidal process, should be identified. The rationale used should be documented, the list should be a controlled document and consistent with that used in validation. Particular attention should be paid to microbiologically retentive filters used to treat services to and from the isolator. When the sporicidal process cannot be assured the use of presterilized filters may be necessary.**
There is a hierarchy of risk leading to direct product contact parts being subject to a conventional sterilization process e.g. filling needles, stoppers etc. Non product contact surfaces including machine surfaces, gloves etc are exposed to a sporicidal process. The indirect product contact surfaces such as stopper hoppers and delivery chutes should ideally be sterilized into the isolator to prepare for the start of each batch of product. Whether it is possible to maintain these surfaces in a satisfactory condition by a sporicidal process for some sequence of batches is unresolved. If the indirect product contact surfaces are exposed to the environment surrounding the isolator by being removed or due to loss of integrity of the isolator, then sterilization is necessary before reuse.

9.4 Sporicidal process (when non gaseous systems are used the relevant principles should still be applied).

9.4.1 **The agent selected for gas generation should be sporicidal.** The agent used for gas generation or other means of application should be capable of rapidly killing bacterial endospores, fungal spores and vegetative microorganisms. Activity against virus, such as is claimed for peracetic acid, may be necessary in some applications or a general advantage. Peracetic acid, hydrogen peroxide and formaldehyde are used. The use of other chemicals such as chlorine dioxide is being developed.

9.4.2 **The correct identity and composition of the gassing agent charged into the gas generator should be assured.** The identity and chemical composition of incoming agent should be assured in the same way as any other critical starting material. The correct dilution and preparation of the agent and filling of the reservoir of the gas generator should be treated as a

critical step in the process and comply with GMP. The storage conditions of the agent should be respected, thus, if refrigeration is required, storage in the gas generator reservoir is not appropriate.

9.4.3 **Gas generators should not be assumed to be equivalent to each other.** The design, operation, validation, maintenance and change control of the gas generator, as an independent piece of equipment, should be treated as critical and comply with GMP. Equivalence of gas generators should not be assumed. Each generator should be shown to deliver an equivalent gassing process to each other, in one or more isolators. If this cannot be demonstrated the gas generators should be treated as different pieces of equipment each with its specific cycle validated for each isolator.

9.4.4 **The way in which the gas generator works should be understood by company staff. All critical parameters related to its operation should be identified and recorded throughout the process. Measuring instruments should be calibrated and where critical should have independent monitors or an assured and confirmed reliability.** Assured and confirmed reliability is a combination of recognized robust design and satisfactory history of operation in the hands of the company.

9.4.5 **The release of the gassing process with regard to the gas generator should verify that all critical parameters met the specifications defined during validation.**

9.4.6 **The delivery of gas from the generator into the isolator should assure that only the gas generated is supplied. All inlet and outlet filters associated with the isolator should be exposed to gas or sterilized. Any air supplied by the generator e.g. during a purge stage, should be filtered though microbiologically retentive filters that have been sterilized or subjected to a sporicidal process.** The delivery of gas to the isolator should be via defined ducts with no possibility of loss or contamination. Dispersed oil droplets used for integrity testing HEPA filters may break down the gas. This should be examined during validation and it may be necessary to consider the first gassing after testing as a neutralising operation. All of the gas should ideally enter by all of the air inlet filters and leave by all of the exhaust filters. If this is not possible arrangements should be made to assure that all terminal inlet and exhaust filters are exposed to gas. If part of the process involves a reversal of the flow through a filter, the possibility of the filter becoming unseated should be investigated.

9.4.7 **The delivery of the correct gas at the validated concentration to the isolator and/or leaving the exhaust system should be confirmed if possible, to supplement the control in 9.4.2.** It is recognized that the technology for such gas analysis is not always available. If this is the case it is even more imperative to assure the steps in 9.4.2.

9.4.8 **The isolator should be cleaned prior to the sporicidal process. The surfaces of packaged materials and all other items to be exposed to the sporicidal process within the isolator should be clean.**
All the surfaces inside the isolator should be clean prior to exposure to the sporicidal process. Apart from removing chemical residues that may contaminate subsequent production, the presence of deposits may enable

microorganisms to survive the process by physical shielding or neutralization of the process of inactivation. The isolator should be designed to enable access to all surfaces for cleaning without major dismantling. Inlet and exhaust air pathways should be designed with this in mind. If clean in place systems are used, any risks that may arise from the presence of spray balls, drains and retained fluids should be identified and eliminated. Whichever cleaning method is used it should result in a visibly clean dry surface free from risk of residues.

9.4.9 **Delivery of gas or other sporicidal treatment to all necessary surfaces should be assured.** All the surfaces inside the isolator system that have to be treated (see 6.4.1) should be exposed to the validated process. There may be some surfaces where access of gas cannot be assured even by using pumps, fans, evacuation, use of point contacts such as round section wire, or other methods. These surfaces should be identified and designed out if possible e.g. by removing or sealing with gaiters, bellows etc. Any remaining surfaces should be minimized and exposed to sporicidal agents e.g. by spraying, wiping, drenching or other means, such that survival of natural occurring microorganisms is unlikely. Direct or indirect product contact with these surfaces should be eliminated.

9.4.10 **The risk of recontamination of sterilized containers leaving the sterilizing zone and entering the cooling zone of a tunnel integrated with an isolator system, should be evaluated. Consideration should be given to sterilization or sporicidal treatment of the cooling zone.**
One area that may be a theoretical risk is the cooling zone of a sterilization tunnel attached to the isolator system. Exposing this zone to hot air sterilization would be ideal otherwise gassing back to the end of the sterilization zone may be possible.

9.4.11 **The sequence of the different aspects of cleaning, sterilization, sporicidal treatment, gassing, and degassing are of critical importance and should be carefully defined and verified before formal release of the system for production.** In a complex system where isolators, sterilizers etc are linked together, the order in which operations are carried out is critical. When one part of the equipment is opened and exposed to adjoining equipment, newly exposed surfaces of the door as well as other surfaces may not be in a compatible state.

9.4.12 **The range of parameters and events that should be monitored to assure the delivery of the validated process should be defined.** The delivery of the validated process will involve monitoring parameters and events in addition to those from the gas generator. These may include the following:

- Gas detection in the isolator/exhaust.
- Gas concentration in the isolator/exhaust.
- Flow rate in exhaust.
- Gas inlet temperature.
- Isolator pressure.

- Pressure drop across filters.
- Condensation detection.
- Temperature of the external surface of the isolator.
- Temperature of internal points in the isolator.
- Absence of alarm conditions.
- Correct operation and position of gas drivers such as fans, pumps and evacuation.
- Displacement to expose occluded surfaces.
- Gas concentration during ventilation.
- Process step times.

9.4.13 **The design, development and validation of the sporicidal process should encompass all relevant aspects from methods of gas distribution to quantification of target lethality, selection, calibration and culture of the biological indicator and definition of the final protocols.** The design, development and validation of a sporicidal process involving gassing should include at least the following steps.

a) Identification of all surfaces that need to be gassed.

b) Selection and validation of the gas agent and generator.

c) Method of distribution of gas to the target surfaces. This may be by mass movement amongst defined loads driven by the gas generator and coupled with passive diffusion along stabilized path lengths. Alternatively, active distribution by fans, pumps, evacuation devices may be employed. Rapid gas cycles (less than two hours exposure to gas, depending on size of isolator) would need careful arrangements of these devices in defined loads to avoid shadowing effects, occluded surfaces etc. If parts of the target surfaces were to be reached by passive diffusion, rapid gas cycles would be unlikely to be effective.

d) As detailed an understanding of the mechanisms for the gassing method chosen, as state of the art allows, is necessary. The effect of variation of all the parameters that may vary and be relevant should be explored during development.

e) An understanding of the relationship between the resistance of the bioburden and that of the biological indicator should be developed from trials and/or the literature.

f) The intended degree of inactivation or lethality can be defined following development trials and based on the information in section e) above. Current practice is to seek six log reductions of the biological indicator organism recommended by the manufacturer of the gas generator. In this document this is intended to mean that at each point in the isolator the sporicidal process will reduce the survivors by six logs i.e. if there are $2x10^6$ spores in the BI to start with then there will be 2 surviving spores after a six log reduction. If there are no survivors, then a six log

reduction is confirmed and there is an additional safety margin the size of which is not known. If there are other ways to verify delivery of the gassing process to all the target surfaces, supported by a well established mechanism of lethality, these may be considered.

g) The carrier type e.g. plastic, paper, metal or other, of the biological indicator organism should be relevant to the materials being gassed or shown to be irrelevant. If studies have been carried to show that lethality on carrier type a is similar to materials c,d,e etc. with a similar sporicidal process, this would mean that in house studies need not be carried out. The data would need to be from a reputable source.

h) The resistance of the biological indicator to the process being validated should be estimated. This can be carried out by plotting the number of survivors against the extent of the process (usually exposure time to the gas). Fraction negative systems may also be used to provide this information. The testing should be carried out in zone that is readily and reproducibly exposed to the process and that is accessible so that biological indicators can be removed from exposure at sequential times to generate a survivor curve. This estimation is to support the requalification when resistance of the biological indicator to be used for requalification is shown to be similar to that used in the original validation, see section 9.4.14 b.

i) The distribution of the gas should be explored using smoke to simulate it or more sophisticated methods to render gas flow visible. Care should be taken to ensure that any residues from these trials that could be trapped on filters or surfaces can be removed or that they will not compromise subsequent gassing or operations, e.g. sulphur trioxide smoke residues break down hydrogen peroxide. Chemical indicators may also be used to track the movement of gas.

j) The BIs should be distributed to sample the full internal volume created by the isolator. In addition positions that are potentially less likely to be exposed to the full gassing process should be tested e.g. areas relying on passive diffusion, areas shadowed from the direct active delivery of gas etc. Continuously occluded surfaces do not qualify for such trials as they cannot be exposed to the process and should have been eliminated, sterilized or subjected to an additional validated process.

k) The details of handling and culture of the BIs should be fully investigated and defined. At the end of the gassing phase there will be a lag as the ventilation reduces the gas concentration. Gas may have absorbed into the material of the BI carrier and into the isolator and load. The desorption of this gas may be difficult to predict. All these factors combine together to produce the potential for residual lethality which may be outside the controlled lethality delivered by the gassing cycle.

When the BI is eventually placed into the tube of broth or carrier medium prior to culture, the gas absorbed in the BI may not be inactivated and could prevent the outgrowth of survivors.

The cultural conditions may not be optimized in terms of media, temperature and time for the outgrowth of survivors. The BI organism may be viable after exposure to the gas, but the recovery system may not be able to allow organisms exposed to the gassing agent to outgrow. The fertility of the particular batch of media used may have varied. All these possibilities should be studied and taken into account in the design of the testing systems.

l) The process of ventilation and degassing should be examined to assure that production is not compromised by outgassing or residues of gas agent in product contact surfaces and materials. For isolators used for sterility testing the absence of traces of residual lethality that could result in a false pass result, should be clearly demonstrated.

m) Once the development work is complete the formal protocols can be defined. These should specify the following aspects as a minimum.

- The gassing process to be validated.
- The condition and loading of the isolator.
- The disposition of specified biological indicators.
- The time at which BIs are to be removed from each position.
- The nature of the recovery medium and details of culture.
- The ventilation and degas phase.
- The acceptance criteria for cycle parameters and BI results.
- The number of repeat studies required.
- The way in which the validated cycle will be enhanced for routine use (usually an additional gassing and ventilation time is added to allow for variation).
- The review and approval process.

9.4.14 **The provisions for requalification and interpretation of results should be carefully and prospectively defined.** The provisions for requalification should be defined in written procedures. These should specify the following aspects as a minimum.

a) The frequency of requalification should involve some repeat of the initial validation work, including degassing, on an annual basis. In addition there should be a program that ensures that all the particular gassing situations and cycles originally validated are requalified within a reasonable time period, which should be no longer than three years.

b) The BIs to be used for requalification should be demonstrated to have a resistance to the gassing process originally validated that is similar to those originally used. This could be carried out by repeating the work described in 9.4.13.h in the same isolator.

c) The details of BI placement, time in the cycle before they are recovered, cultural conditions etc. These should repeat the relevant aspects of the original validation without applying safety margins employed during routine gassing cycles.

d) The evaluation of results should recognize that validations using BIs may not be able to be exactly repeated due to the inherent uncertainties surrounding biological systems. It is for this reason that large safety margins are added. The process that delivers a six log reduction of the BI should be in excess of that necessary to kill the bioburden that can reasonably be anticipated. This, together with the additional gas exposure time applied routinely provide these safety margins.

9.4.14.1 Against the background of uncertainty described in 9.4.13d, the requalification and evaluation of results should be able to provide quantitative information about the actual log reductions found at each point in the isolator where BIs are placed. There are a variety of ways that this may be carried out. One BI may be placed in each position and be subjected to a process of washing off the spores or dispersing the BI followed by plate culture and counting of colonies, or culture of aliquots of the spore suspension to give a most probable number estimation of survivors. If there are survivors the number of log reductions can be calculated. Alternatively, if two BIs are placed at each position and one is refrigerated (storage would need validating) and the other placed in broth for incubation, a no growth from the broth gives a clear result. If there is growth in the broth then one or more survivors are present, the remaining BI can then be analysed as above to determine the number of survivors and enable the log reductions to be calculated. Another possibility is to place three or more BIs at each position in the isolator and put them individually into broth for incubation. If there are any positive broths the proportion of positive to negative can be used to estimate the number of survivors and thus the log reductions. Given this information any variation in the process is estimated and the significance of it can be evaluated. If there is only one BI in each position, and only growth/no growth is established, then the number of any survivors is unknown and the size of the possible variation in the process cannot be estimated.

9.4.14.2 The significance of variation should take into account that gassing is an environmental control process that is at least one step removed from the control of product sterility. For example, if a variation at one position in the isolator resulted in only four log reductions being demonstrated, the cause of variation should be investigated and corrected, or if the cause cannot be found the safety margins added to specify the routine cycles used may need to be increased. The effect of the variation on past production will depend on the cause of the variation. If the routine delivery of four logs plus the safety margin, can reasonably be expected to reduce actual bioburden to a level where survivors would not be expected, it may be reasonable to conclude that production has not been compromised.

9.5 The prevention of recontamination

9.5.1 **All gases, fluids and air supplied to the isolator or that may gain access, should be filtered using microbiologically retentive filters or sterilized prior to entry**.
All gases and fluids passing into the isolator should be filtered using microbiologically retentive filters or sterilized prior to entry through the envelope so that any escape inside the isolator will be of uncontaminated material. Any vacuum points should be guarded by filters. Consideration should be given to providing a HEPA prefilter for the air inlet system mainly to provide redundancy in the event of failure of one of the filters. The duty to exclude penetration by microorganisms in the incoming air is probably higher than for conventional clean rooms as discussed in 9.1. The air that is supplied to the critical zone of a conventional clean room is generally double HEPA filtered i.e. once into the room and again into the Grade A zone. HEPA filtration is not absolute and a rare penetration is to be expected. The main intention is to provide redundant filtration because if only single filtration is used in the isolator a filter failure could increase the risk of contamination significantly. HEPA filtration of the exhaust system is a standard precaution against backflow.

9.5.2 **The control of leaks between the isolator and surrounding room and between different parts of the isolator system as necessary, should be assured as far as possible. As a guide a minimum of 10 Pascal positive differential air pressure should be maintained to protect against unforeseen circumstances. The maintenance of positive pressure should be monitored and fitted with an alarm**. The isolator should be designed to be free from leaks that are a microbiological risk and maintained in that state. It is recognized that there will be some leakage, but this should be due to essential engineering tolerances as opposed to poor design, construction and maintenance. There should be a program to reduce the risk of leaks due to accident and means of detecting them which have known sensitivity e.g. pressure hold tests, tracer gas penetration etc. The risk posed by undetected leaks and unanticipated deterioration can be reduced by operating the isolator at positive pressure with respect to lower grade connecting and surrounding areas. A pressure sufficient to maintain a differential of at least 10Pa under all operating conditions is suggested. If requirements for operator safety drive the need to use a negative pressure critical zone, consideration should be given to enclosing it in a positive pressure envelope.

9.5.3 **A program to minimize the risk of loss of integrity of gloves, sleeves and suits should be present. This should include operator practices, vigilance and the absence of sharp edges. There should also be an all encompassing preventative maintenance program that includes specification of examination and preemptive replacements.**

9.5.3.1 Glove ports and full or half suits present particular risks for the following reasons:

- They are more prone to damage.
- They may be very close to exposed sterile materials.

- They may not be protected by positive pressure due to localized sealing effects, the piston effect of arms entering the sleeves and occlusion.
- The air and surfaces exposed by the leak may be microbiologically contaminated due to the proximity of the operator's body.

9.5.3.2 The analysis of these risks should be documented and preventative actions such as the following should be considered:

- Selection of robust materials.
- Use of double skinned sleeves where puncture of one or both of the skins causes separation of the two layers and is easily detected by the operator.
- Operator training to avoid damage and vigilance to examine for damage.
- Frequent leak testing.
- Inner or outer sterile gloves.
- Sterile inner sleeves or garments.
- Preventative maintenance program that includes specification of examination and preemptive replacement

9.5.3.3 The use of aseptic techniques, to the extent possible in isolators, provides additional reduction in the risk to product arising from loss of integrity of sleeves and gloves.

9.5.4 Transfer of material out

Transfer of material out of the isolator should not compromise the critical zone. Product and waste should ideally be removed from the isolator without loss of integrity. Alternating gassed accumulation airlocks or heat sealed sterile plastic film tube may be applicable depending on the scale of production. When it is impossible (as opposed to inconvenient) to provide a continuous gassed/sterilized/ physical barrier, the opening should be properly designed. The design should ensure that the opening should be able to be sealed during gassing or when left unattended. During use, the design should provide robust methods of preventing penetration by the use of, for example, directional airflow, transition chambers or tunnels and distance from the critical zone. When transfer out involves transition to another aseptic stage such as a lyophilizer connected to the isolator system, the transfer should assure the integrity of the isolator as well as the safety of the product.

9.5.5 Transfer of material in

Transfer of material into the isolator should not compromise the critical zone. Materials to be transferred into the isolator should comply with 6.4.1 to avoid them carrying contamination into the isolator once it is in its gassed state. Transfer to the isolator should be minimized and secure to prevent

penetration of contaminants during the transfer process[1]. Examples of transfer in scenarios are as follows:

- Secure transfer ports from a separate autoclave, isolator, or supplier of sterile components, formulated drug powder etc. Any small area of the gasket that has been exposed to the external environment and is then exposed to the inside of the isolator should be managed (this includes the 'ring of concern' of rapid transfer ports). This may include manual surface sanitisation, or use of heat or light sporicidal processes coupled with no direct or indirect product contact.
- Direct connection between the isolator and other isolators, autoclaves, hot air ovens, sterilizing tunnels etc. The interfaces should be carefully designed to withstand the stresses of extreme temperatures, expansion and contraction and retain the integrity of the isolator system. When intervening doors are opened, there should not be any exposure of non-sterile or non-gassed surfaces or ingress of unfiltered air. Ingress of steam and condensate from an autoclave should be prevented.

9.5.6 Air change, laminar/turbulent, aseptic technique, and ergonomics

The design of the isolator system should include consideration of air change rate, the use of laminar, unidirectional or turbulent airflow, the application of aseptic technique and the risk of error due to human fallibility. The rationale for the decisions taken should be documented.

9.5.6.1 The air change rate should be sufficient to ventilate the operation avoiding build up of aerosols, powder, packaging particles and flushing away microorganisms in the unlikely event they are present.

9.5.6.2 As the absence of microorganisms is expected the questions of laminar flow versus turbulent flow and the rigour of implementation of aseptic procedure may be irrelevant. During the design of isolators it may be as convenient to arrange for incoming air to be delivered to form a laminar or unidirectional flow or as a turbulent flow. The lower air velocity generated by the laminar or unidirectional option may reduce risks of venturi effects and impacts on production operations. In these cases, it would seem sensible to also gain another increment of sterility assurance and arrange airflows and production operations accordingly.

9.5.6.3 On the occasion that manual operations are carried out it again seems sensible to gain another increment of sterility assurance and act as if gloves are contaminated and to use aseptic techniques to the extent possible in isolators.

9.5.6.4 The engineering and procedural arrangements to prevent recontamination and generally to secure production may be neutralized by mistakes by the operators. Isolator systems are not the same as clean rooms and different types of error are possible. The system should be designed and operated with due regard to human fallibility.

---

[1] The special problem of the cooling zone in a sterilizing tunnel has been discussed above.

9.5.7 Monitoring and testing

9.5.7.1 **Physical monitoring and testing should be based on a systematic failure mode analysis or a suitable alternative and assure the detection of change, failure or aging that could compromise operations.**

9.5.7.1.1 The main thrust of control of this type of isolator is physical; therefore, physical monitoring and testing is preeminent. The testing should be organized to monitor the parameters considered to be critical together with their alarm systems. Alarms should be latched so that the occurrence of the alarm is still evident even though the deviation leading to the alarm being triggered has corrected itself. This is valuable when the isolator is left unattended e.g. at night. The following should be considered.

- Isolator pressure.
- Airflow in. Airflow out.
- Pressure change across filters.
- Temperature/Humidity-depends on process.
- Airborne particles, the position of the sampling probe for continuous monitoring should be carefully considered. If it is positioned just to sample a limited output of the air inlet filter it is unlikely to provide useful information. Sampling near the point of fill, in the recirculation ducts or exhaust ducts may be more informative. When powders are handled the sampling program should select times and positions to provide relevant information.

9.5.7.1.2 The following tests and programs should be considered:

- Leak testing.
- Systematic visual examination.
- Filter integrity.
- Calibration.
- Maintenance checks of structure as well as equipment.

9.5.7.2 **Microbiological monitoring should take into account the special requirements for sensitivity of testing in isolators subjected to a sporicidal process and avoid compromising operations. The interpretation of results of environmental monitoring should be based on the premise that the detection of any microbiological contamination probably indicates a failure of the system.**

9.5.7.2.1 **Media fills and sterility testing should be carried out as normal for aseptic processing.**

9.5.7.2.2 Environmental monitoring within the isolator should not interfere with zone protection, and in process controls should not carry any risk for production.

9.5.7.2.3 The use of settle plates, contact plates, swabs and the presence of sampling points for active air samplers or particle counters may add risk to the system subjected to a sporicidal process. Some of the ways that this may be addressed include the following:

- Sampling at the end of production.
- Sampling at potentially worst case positions e.g. in an exhaust.
- Using multiple wrapped irradiated plates and swabs etc. may reduce the risk of introducing contamination into the system, but there have been instances when the supplier has made changes or mistakes and compromised processes. The fertility of irradiated media should be given special attention. Testing the supplier's formula at extremes of the irradiation treatment using local isolates as well as standard cultures should be considered. The effect of exposure of wrapped plates etc. to the sporicidal process should be examined in case of loss of fertility due to penetration of the agent.
- A significant risk to the interpretation of results is the accidental infection of plates etc. by subsequent handling, so incubation in sealed sterile pass out bags may be necessary. Another risk to the interpretation of results is the presence of a colony that developed prior to irradiation.
- Built in sampling systems should be gassed or otherwise assured to be free from contamination and not compromise operations, special arrangements of filters and/or valves may be used.
- Quantitative results are not as relevant as in conventional clean rooms because the detection of any contamination probably indicates something has failed. Conventional sampling may be replaced by 'in house' devices known to be sterile, such as settling pots full of media or transport fluid. Large areas of the gloves and isolator surfaces may be swabbed and the swab incubated in sterile broth.

9.5.7.2.4 **Evaluation of results**

- The detection of any microorganisms from environmental monitoring inside the isolator should be considered as requiring a full scale investigation. Consideration should be given to the wisdom of releasing product still in house and the continued use of the isolator may not be appropriate.

- If a clear cause is found, the implications on existing product could be evaluated based on the likely level and type of environmental contamination, together with the probability of contamination of product. Loss of integrity of gloves, mistakes in transfer of materials into the system, and contaminated settle plates have been implicated as causes based on past experience. If no clear cause is found after a genuinely searching investigation; and intensive monitoring shows no further contamination; this may be a case when the slight imperfections of the system are revealed, and as long as they do not reoccur they may have to be accepted.

- A positive media fill unit or positive sterility test unit is a more serious event and the effects on product in the field may have to be considered as there is some evidence of non-sterile product being produced and supplied.

- In this case the investigation would not only involve possible failure of the isolator to control the environment, but the whole sterility assurance system including components, formulated drug sterilization, on site and any off site sterilization processes, product integrity etc. It is only when the cause is found that appropriate action can be taken.