DESIGN, OPERATION, QUALIFICATION AND MAINTENANCE OF ISOLATOR

Review Article

ABSTRACT

Technology transformed for aseptic process from conventional clean room concept to processing isolator technology. This review article focus on the Design, Operation, Qualification and maintenance aspects of Aseptic processing Isolator system

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This article does not necessarily represent the opinion of the author nor employers.

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Contents

Review Article

Design, Operation, Qualification and maintenance of Isolator

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Isolators have been around the Pharmaceutical Industry since the early 1980s and in the Nuclear Industry (glovebox technology) since the 1950s. The intent of isolators is to create an airtight barrier or enclosure around a piece of equipment or process which provides absolute separation between the operator and product. The operator can perform tasks through half-suits or glove ports. Isolators provide a specific environment inside the isolator using HEPA filters. The environment can be positive pressure or negative, can have humidity control, oxygen control, use unidirectional airflow, and can either protect the product from the operator as with aseptic processes, or protect the operator from the product as with potent product handling. The earliest uses of aseptic isolators were for sterility testing. Sterility test isolators make up most of the aseptic isolators in use and are available in many different sizes and configurations. Sterility test isolators do not need to be installed in a classified area. No formal requirement exists for a Grade D environment, but the area should be controlled to allow only trained personnel. The room should also have temperature and humidity control.

1. Applications

People are the greatest source of contamination in the manufacture of sterile products. Over the past decade, substantial progress has been made in separating the operator from the critical areas within the aseptic manufacturing suite. Isolators, RABS, blow-fill-seal, conventional barriers, and the increasing use of robotics in these systems have increased personnel separation from the critical areas. Many of the advantages of these technologies, however, can be negated by poor design, lack of knowledge concerning their operation, and ineffective operator training. Absolutely basic to the design concept are the ergonomic aspects of the production operation to be undertaken. This should be considered in conjunction with mechanical movement, and appropriate material and equipment transfers, sterilizability, and an appropriate background environment in which the system is to be operated. These decisions should be made on a case-by-case approach, depending upon the application and specific system design. Aseptic processing presents a higher risk of microbial contamination of the product than terminal sterilization Aseptic processing presents a higher risk of microbial contamination of the product than terminal sterilization closures are sterilized separately and

then brought together under an extremely high quality environmental condition designed to reduce the possibility of a non-sterile unit.

Aseptic processing involves more variables than terminal sterilization. Any manual or mechanical manipulation of the sterilized drug, containers, or closures prior to or during aseptic filling and assembly poses the risk of microbial contamination.

Some types of aseptic processing involve manual manipulations of sterile components, containers, and closures in addition to routine operator interventions in the critical area. Humans are a significant source of contamination in traditional aseptic processing, especially in production lines that require operators to routinely enter critical areas (Class 100, ISO 5, or Grade A) of the filling line. Aseptic processing systems based on more advanced control-based technologies, such as Restricted Access Barrier Systems (RABS) and Blow-Fill-Seal systems, are designed to reduce human interventions in the critical areas of the fill line while an isolator system completely separates the aseptic filling line from the external environment and minimizes employee interaction with the critical area

1.1 Isolator:

The design and control elements that maintain the separation or isolation of the product. Pressure differential, glove integrity, and protection of the transfer (i.e., entry, exit) ports are key elements for the isolators. The transfer of containers, closures and supplies (including environmental monitoring supplies) into an isolator should be carefully controlled. Another critical element for these systems is the effectiveness of the chamber decontamination program. Current methods (e.g., vaporized hydrogen peroxide, steam hydrogen peroxide, peracetic acid) used to decontaminate isolator barriers are capable of surface sterilization but lack the penetrating capabilities of steam sterilization.

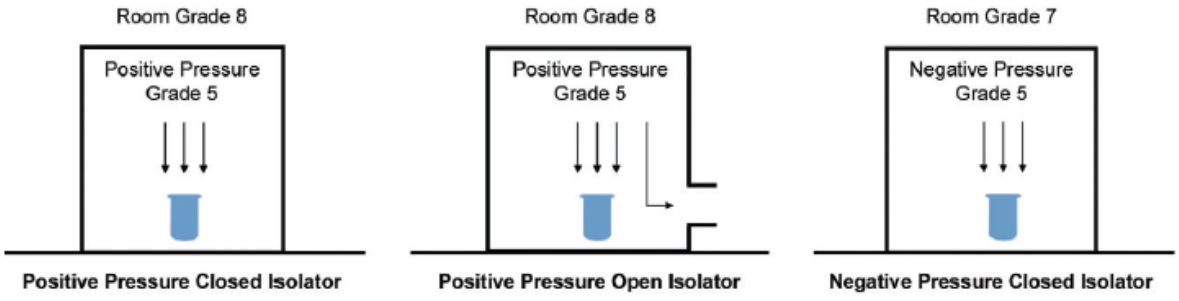


Figure: Isolator Types and Surrounding Environment Classifications

Investigators should be mindful of the limitations of these surface sterilants, including their inefficiency in penetrating obstructed or protected surfaces. Validation of the decontamination of the interior (surfaces) of an isolator should demonstrate a 6-log reduction of the biological indicator (BI). Quantitative measuring devices (e.g., near infrared) or chemical indicators (qualitative test) can be used to determine the worst case location for decontamination

validation using BI. Factors to be considered in decontamination validation include the location of the BI and the type of surfaces where the BIs are inoculated.

Utensils and equipment surfaces inside the isolator that have direct contact with sterile product and components should be sterilized to render them free of microorganisms. The sterilization validation should achieve a minimum of a 6-log reduction of the BI.

1.2 Restricted Access Barrier System (RABS):

In general, a RABS is a fill-finish line in a rigid wall enclosure that provides full physical separation of the filling line from operators. It is important to note that the inside surfaces of the RABS are disinfected with a sporicidal agent, but this is not accomplished using the automated decontamination cycles employed for isolators. This requires firms to carefully supervise disinfection procedures and assure ongoing effectiveness of the disinfection program. Operators use glove ports, half suits or automation to access areas within the enclosure during filling. There are 2 types of RABS, "open" and "closed" RABS. The doors to a "closed" RABS are never opened during an operation. While an "open" RABS is designed to operate with doors closed at all times, on rare pre-defined circumstances the doors of the enclosure can be opened to perform certain interventions. If doors are routinely opened during a filling operation, the system is not considered a RABS because it no longer restricts access to the critical areas. Typically, the clean room surrounding the RABS is controlled as a Class 10,000 (ISO 7) area and operators are fully gowned.

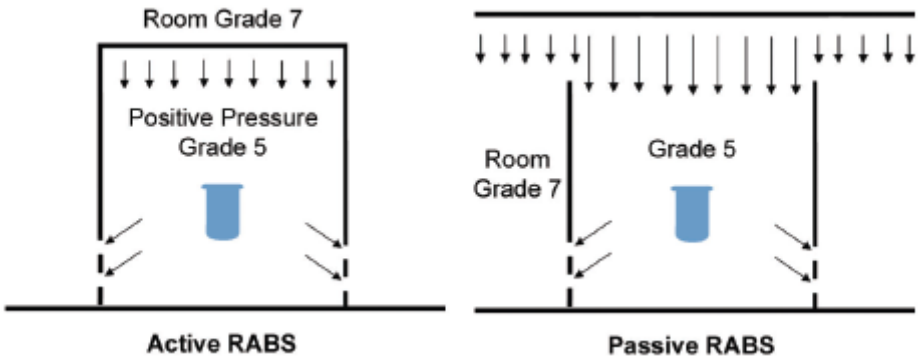


Figure: Active and passive RABS and Surrounding Environment Classifications

1.3 Media fills or process simulations:

Media fills are used to validate aseptic processing operations, including those employing newer technologies, such as isolators, BFS or RABS systems. Media fills representing manually intensive aseptic operations should equal or approach the size and duration of a commercial production lot. In contrast, a process conducted in an isolator is designed to have a lower risk of microbial contamination because of the lack of direct human intervention and can be simulated with a lower number of units as a proportion of the overall operation. All media fills

should closely simulate manufacturing operations, incorporating, as appropriate, worst-case activities and conditions as well as operator interventions.

Determine if media fills are conducted semi-annually for each processing line. The activities and interventions representative of each shift should be included in the semi-annual media fill program. This may require more than one media fill per line every 6 months, if aseptic processing is performed during more than one shift. With the exception of isolator operations, at least one semi-annual media fill is performed per line per shift. Determine if the aseptic filling of all types of containers are supported by the media fills performed. If a matrix approach is used, robust scientific justification is required for selecting the worst case container / closure configurations for each line.

2. Design

Maintenance of isolator systems differs in some significant respects from the traditional, non isolated aseptic processing operations. Although no isolator forms an absolute seal, very high integrity can be achieved in a well-designed unit. However, a leak in certain components of the system can constitute a significant breach of integrity. The integrity of gloves, half-suits, and seams should receive daily attention and be addressed by a comprehensive preventative maintenance program. Replacement frequencies should be established in written procedures that ensure parts will be changed before they breakdown or degrade. Transfer systems, gaskets, and seals are among the other parts that should be covered by the maintenance program.

Isolators can be either "open" or "closed" depending upon their operational state and may operate at positive, neutral, or negative pressures with respect to the surrounding environment. When "closed," isolators may exchange air with the surrounding environment only through microbially retentive filters. When "open," isolators may transfer air directly to the surrounding environment through openings (e.g., "mouseholes") that preclude the ingress of bio-contamination. Following are the key factors which we should look into,

- Materials of Construction
- Manual vs Automatic Valves
- PLC Control System
- Panel HEPA Filters for Aseptic Applications
- Airflow/pressure differential/air classification
- Oversize Blowers for rapid aeration
- Glove Integrity
- Trash Receptacle and Evacuation of Filtrates
- Internal Fans for Sterilant Distribution
- Transfer of Materials/supplies
- Decontamination
- Ports for Environmental Monitoring
- Strategic Location of Gloves and Half-Suits
- Shelving for Storage
- Built-In Steritest System
- Rapid Transfer Ports (alpha-beta doors)
- Pass-Through Connections for Service
- Mechanical Aids for Transferring Materials
- Glove Supports During Decontamination
- Autoclave, Oven and Freeze Dryer Interface (if any)
- Integration with vH2O2 Generators providing fully automatic decontamination cycles

Surface Finishes

Passivation may be required to prevent corrosion of ferrous metals contaminated by vapor-phase hydrogen peroxide gases. The isolator surface should be finished to a uniform dull polish, generally No. 4 (240 grit) or better. All chamber joints should be fully welded, hygienic construction, crack- and crevice-free with generously radiused corners for easy cleaning.

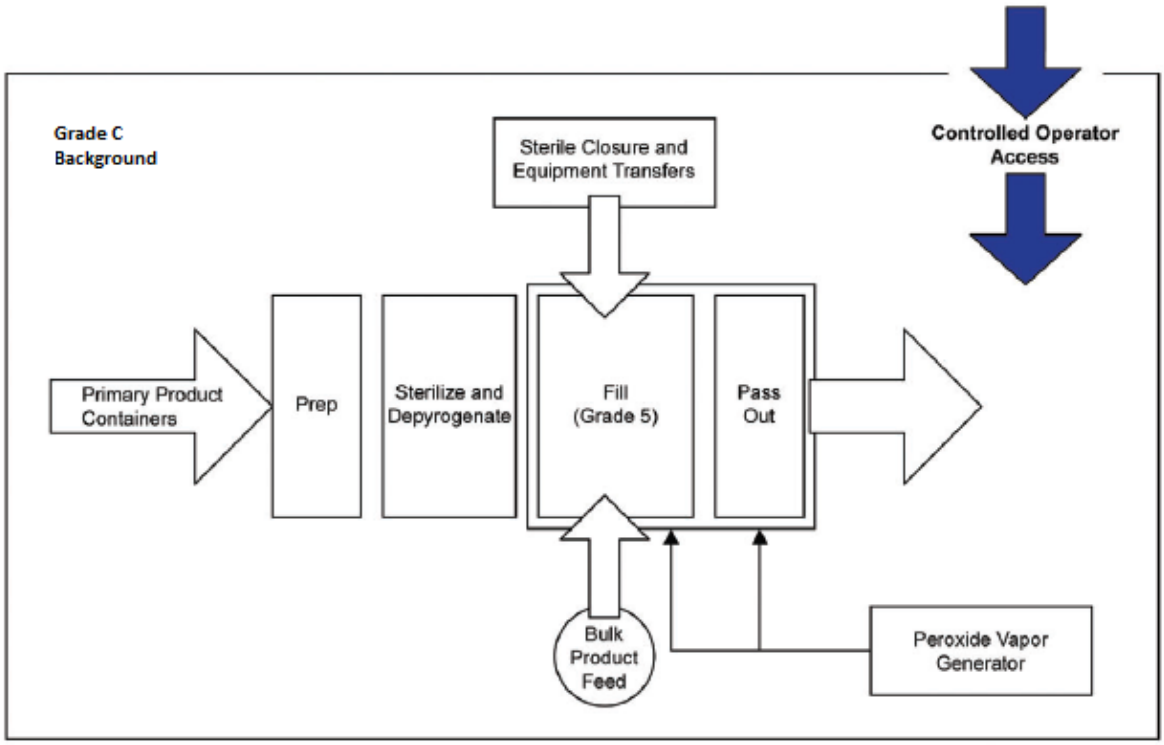


Figure: Example – Arrangement for an Isolator Facility

3. Component and equipment transfer

The ability to maintain integrity of a decontaminated isolator can be affected impacted by the design of transfer ports. Various adaptations, of differing capabilities, allow for the transfer of supplies into and out of the isolator. Multiple material transfers are generally made during the processing of a batch. Frequently, transfers are performed via direct interface with manufacturing equipment. Properly maintained and operated rapid transfer ports (RTPs) are an effective transfer mechanism for aseptic transfer of materials into and out of isolators. Some transfer ports might have significant limitations, including marginal decontaminating capability (e.g., ultraviolet) or a design that has the potential to compromise isolation by allowing ingress of air from the surrounding room. In the latter case, localized HEPA-filtered unidirectional airflow cover in the area of such a port should be implemented. Isolators often include a mousehole or other exit port through which product is discharged, opening the isolator to the outside environment. Sufficient overpressure should be supplied and monitored on a continuous basis at this location to ensure that isolation is maintained.

H2O2 vapour bio-decontamination tunnels have been developed for decontamination transfer of ready to-use sterilised containers for entry into filling isolators. H2O2 is a strong oxidising agent with proven efficacy on bacteria, fungi, spores and viruses. H2O2 vapour is an Environmental Protection Agency registered agent due to its broad spectrum efficacy and its ability to rapidly inactivate the most resilient microorganisms. H2O2 vapour has proven biological efficacy against a wide range of microflora, including bacterial endospores and fungi.

Typically, the decontaminating vapour is generated by flash evaporation from 35% (wt/wt) liquid H2O2. The H2O2 vapour disinfection 'kill' process action is via free radical attack . Free radicals are highly energetic atoms or molecules that possess an unpaired electron. The unpaired electron is compelled to exist in a paired state and will physically strip another compound to promote pairing. Since there is no resistance to free radical attack, free radicals can, on exposure to a range of compounds including organic matter, give rise to the formation of the highly reactive hydroxyl (OH) which is known to have a very high oxidisation potential and low specificity resulting in broad spectrum efficacy.

H2O2 as a powerful oxidising agent has the process compatibility attribute to break down to harmless components of oxygen and water in a residuals removal step called aeration. The H2O2 bio-decontamination process occurs at relatively low temperature (30ºC) and is residue free. It should be noted that gas molecules will pass through the tyvek™ tub cover with slow back diffusion so there is H2O2 contact with product containers. This, however, needs to be studied as product containers are exposed to the oxidising agent. Even traces of residue can impact biological products if retained. Biological indicators are used to qualify the efficacy of decontamination cycles on surfaces at a defined sporicidal log reduction, including H2O2

vapour. Decontamination processes are automated to provide the necessary repeatability and robustness to ensure material transfers of tubs with tyvek™ covers are free of contamination in the transfer process to prevent subsequent contamination of the isolator Grade A environment where tyvek™ covers (lid and liner) are removed just after entry and product containers are then exposed and at risk of contamination.

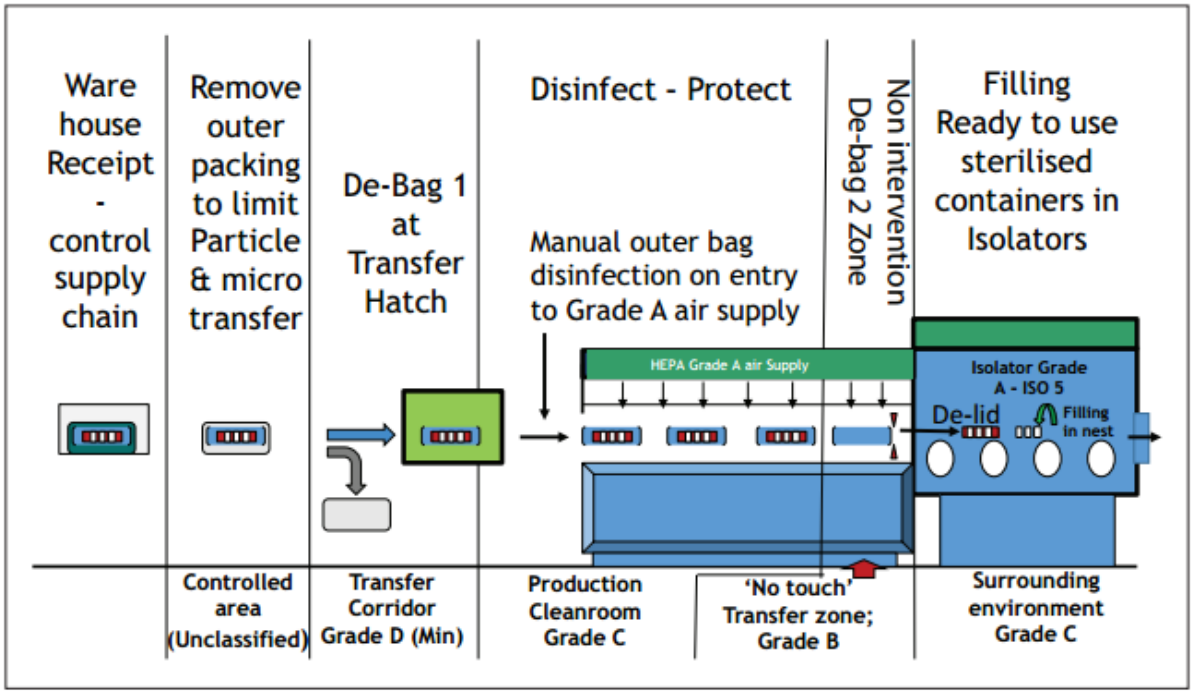


Figure: Ready-to-use sterilised container transfer from receipt to use in filling lines via de-bagger and 'no-touch' transfers.

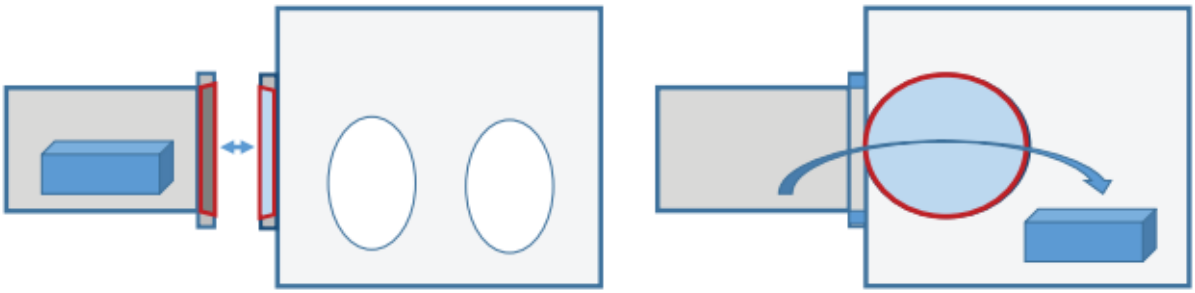


Figure: Principle of RTP 'Alpha – Beta' door closed aseptic transfers

A common technique for passing items into the enclosure is via a Rapid Transfer Port (RTP). In this case, items are sterilized in a separate canister, which is designed to be docked onto the transfer door of the enclosure. The docking process seals the outer face of the transfer door to the lid of the canister in an air tight manner. Air tightness is ensured by the use of multiple-lip seal gaskets. The action of locking the canister to the enclosure simultaneously releases the lid from the canister and locks it onto the transfer door of the enclosure which can then be opened from inside using the glove access. The seals should be sterilizable and frequently high-level disinfected. The number of times RTPs are used should be minimized, as each use

increases the probability of contamination. Where an RTP is not practical, the interface should be sterilizable.

Maintenance of the RTP port and multiple-lip seal gaskets is critical to preclude contamination.

Design should permit passage of items to and from the enclosure without opening it to the surrounding area. In the cases where the transfer system for the enclosure has to be open to the surrounding room, e.g., mouse-hole exits or where it is integral with a dry heat tunnel sterilizer for the transfer of components, the direction of airflow must ensure that contaminants will not pass into the enclosure, and that appropriate DPs are maintained and local protection afforded.

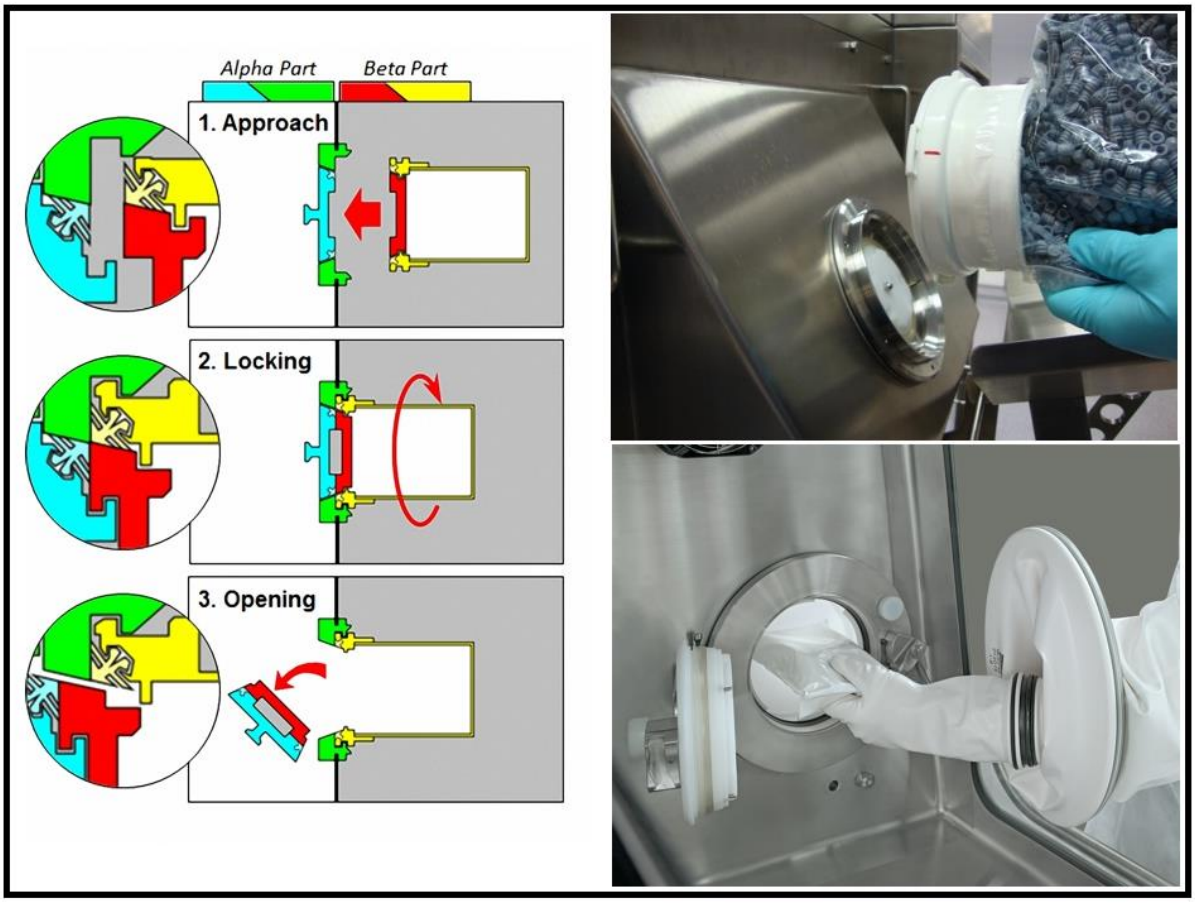


Figure: Rapid Transfer Port operation mechanism

4. Cleaning, Decontamination & Disinfection

Prior to beginning decontamination, the isolator should be mechanically cleaned to remove contamination that may otherwise interfere with the effectiveness of the surface decontaminant. Both the cleaning technique and cleaning process must be validated, so engineers should consider the ergonomics of access, via the glove ports, or half-suit, to all surfaces within the isolator if the isolator is to be cleaned while closed. The use of extension tools for cleaning some internal surface areas may be required.

A properly designed and validated vapor treatment to decontaminate the isolator should be implemented. It should be noted that only surface decontamination is accomplished by the various treatments that may be used. Surfaces must be exposed sufficiently to the agent in order to achieve isolator decontamination. The isolator decontamination cycle should be validated to ensure its effectiveness throughout the isolator.

The necessary level of decontamination should be determined on the basis of risk assessment and analysis.

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.

Figure: Isolator connected with VHP Gas generator

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. 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.

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.

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

Removal of the Decontaminant – Aeration

Some hydrogen peroxide generators use internal catalytic converters to remove breakdown products of peroxide during decontamination, replenishing it with fresh vapor to maintain the decontamination process. This catalyst also is used to remove residual hydrogen peroxide from the isolator at the end of the cycle. In larger systems, this converter can be external to increase aeration capacity.

Note: Catalytic converters may not be required or needed in all cases.

Peroxide vapor should be removed from the chamber at the end of the cycle to prevent it from venting into the workplace or contaminating the product. The normal return air breakdown from the generator may be supplemented by additional air handling to purge the isolator. This purge air exhaust normally is protected by a catalytic converter (usually platinum on alumina). Partial aeration of the isolator, using the catalyst in the generator, may be an alternative prior to venting the isolator to the atmosphere. Local environmental regulations, however, should be considered before exhausting peroxide from a partially aerated isolator to the atmosphere.

Verification of the effectiveness of purge vapor catalysts is generally assessed by sampling the downstream airflow for absence of vapor, using commercially available vapor detection tubes.

Aeration times should compensate for absorption of the vapor on the surfaces, e.g., vinyl, PVC of gloves and half suits, and into packing materials, e.g., the Tyvek™ paper of equipment wraps, also glazing gaskets, product tubing, and HEPA filter media. The aeration time is prolonged in these instances. The use of suit supports or glove extenders will reduce folds where vapor can be trapped. After aeration to levels below 1 ppm residual vapor-phase hydrogen peroxide, it is possible that continued desorption from polymers will contribute to airborne vapor levels.

Upon completion of the surface decontamination phase, and during the aeration phase, it is vital to design the air handling to maintain DP up to, and including, the point that normal airflows are re-established.

Surface Exposure

Decontamination procedures should ensure full exposure of all isolator surfaces to the chemical agent. The capability of a decontaminant to penetrate obstructed or covered surfaces is limited. For example, to facilitate contact with the decontaminant, the glove apparatus should be fully extended with glove fingers separated during the decontamination cycle. It is also important to clean the interior of the isolator per appropriate procedures to allow for a robust decontamination process.

Efficacy

The decontamination method should render the inner surfaces of the isolator free of viable microorganisms. Multiple available vaporized agents are suitable for achieving decontamination. Process development and validation studies should include a thorough determination of cycle capability. The characteristics of these agents generally preclude the reliable use of statistical methods (e.g., fraction negative) to determine process lethality (Ref. 13). An appropriate, quantified Biological Indicator (BI) challenge should be placed on various materials23 and in many locations throughout the isolator, including difficult to reach areas. Cycles should be developed with an appropriate margin of extra kill to provide confidence in robustness of the decontamination processes. Normally, a four- to six-log reduction can be justified depending on the application. The specific BI spore titer used and the selection of BI placement sites should be justified. For example, demonstration of a four-log reduction should be sufficient for controlled, very low bio- burden materials introduced into a transfer isolator, including wrapped sterile supplies that are briefly exposed to the surrounding cleanroom environment.

The uniform distribution of a defined concentration of decontaminating agent should also be evaluated as part of these studies. Chemical indicators may also be useful as a qualitative tool to show that the decontaminating agent reached a given location.

Frequency

The design of the interior and content of an isolator should provide for its frequent decontamination. When an isolator is used for multiple days between decontamination cycles, the frequency adopted should be justified. This frequency, established during validation studies, should be reevaluated and increased if production data indicate deterioration of the microbiological quality of the isolator environment. A breach of isolator integrity should normally lead to a decontamination cycle. Integrity can be affected by power failures, valve failure, inadequate overpressure, holes in gloves and seams, or other leaks. Breaches of integrity should be investigated. If it is determined that the environment may have been compromised, any product potentially impacted by the breach should be rejected.

Filling Line Sterilization

To ensure sterility of product contact surfaces from the start of each operation, the entire path of the sterile processing stream should be sterilized. In addition, aseptic processing equipment or ancillary supplies to be used within the isolator should be chosen based on their ability to withstand steam sterilization (or equivalent method). It is expected that materials that permit heat sterilization (e.g., SIP) will be rendered sterile by such methods. Where decontamination methods are used to render certain product contact surfaces free of viable organisms, a minimum of a six-log reduction should be demonstrated using a suitable biological indicator.

5. Physical Monitoring

Although cleanroom apparel considerations are generally reduced in an isolator operation, the contamination risk contributed by manual factors cannot be overlooked. Isolation processes generally include periodic or even frequent use of one or more gloves for aseptic manipulations and handling of material transfers into and out of the isolator. One should be aware that locations on gloves, sleeves, or half suits can be among the more difficult to reach places during decontamination, and glove integrity defects might not be promptly detected. Traditional aseptic processing vigilance remains critical, with an understanding that contaminated isolator gloves can lead to product non sterility. Accordingly, meticulous aseptic technique standards must be observed, including appropriate use of sterile tools for manipulations.

6. Leak Testing

The type of isolator and its design will determine leakage characteristics (e.g., number of windows, glove ports, and transfer ports). Isolator leakage is of concern where there is potential for loss of decontaminant gas or toxic product, or where it may allow the air from the surrounding environment to enter via induction during glove and half-suit entries and exits.

The leak test is designed to ensure that the isolator continues to be operated within its original design characteristics.

Although gas leakage can be detected, small leaks may not be detected. The method of leak testing should be defined at the design stage and an acceptable leak rate should be established between the manufacturer and the user. A number of different leak test methods are possible:

- Leak test by pressure drop
- Leak test by maintaining a constant pressure with a known flow rate
- Leak test using a tracer vapor
- Ultrasonics, etc.

Alternative tests could be developed which may be equally appropriate for a particular design. A relevant leak test specification should be established as part of the maintenance program.

Glove Integrity

A faulty glove or sleeve (gauntlet) assembly represents a route of contamination and a critical breach of isolator integrity. A preventative maintenance program should be established. The choice of durable glove materials, coupled with a well-justified replacement frequency, are key aspects of good manufacturing practice to be addressed. With every use, gloves should be visually evaluated for any macroscopic physical defect. Physical integrity tests should also be performed routinely. A breach in glove integrity can be of serious consequence. The monitoring and maintenance program should identify and eliminate any glove lacking integrity and minimize the possibility of placing a sterile product at risk.

Due to the potential for microbial migration through microscopic holes in gloves and the lack of a highly sensitive glove integrity test, we recommend affording attention to the sanitary quality of the inner surface of the installed glove and to integrating the use of a second pair of thin gloves.

7. Microbiological Monitoring

An environmental monitoring program should be established that routinely ensures acceptable microbiological quality of air, surfaces, and gloves (or half-suits) as well as particle levels, within the isolator.

Nutrient media should be cleaned off of surfaces following a contact plate sample. Air quality should be monitored periodically during each shift.

Written procedures should include a list of locations to be sampled. Sample timing, frequency, and location should be carefully selected based upon their relationship to the operation performed.

All environmental monitoring locations should be described in SOPs with sufficient detail to allow for reproducible sampling of a given location surveyed. Written SOPs should also address elements such as (1) frequency of sampling, (2) when the samples are taken (i.e., during or at the conclusion of operations), (3) duration of sampling, (4) sample size (e.g., surface area, air volume), (5) specific sampling equipment and techniques, (6) alert and action levels, and (7) appropriate response to deviations from alert or action levels.

	Maximum permitted number of particles per m^3 greater than or equal to the tabulated size			
	At rest[a]		In operation[b]	
Grade	**0.5 µm**	**5.0 µm**	**0.5 µm**	**5.0 µm**
A	3 520	20	3 520	20
B	3 520	29	352 000	2 900
C	352 000	2 900	3 520 000	29 000
D	3 520 000	29 000	Not defined	Not defined

Figure: Maximum permitted airborne particle concentrate

Grade	Air sample (CFU/m3)	Settle plates (diameter 90 mm) (CFU/4 hours)[b]	Contact plates (diameter 55 mm) (CFU/plate)	Glove print (5 fingers) (CFU/glove)
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

CFU, colony-forming units.
[a] These are average values.
[b] Individual settle plates may be exposed for less than 4 hours.

Figure: Recommended limits for microbial contamination

8. Validation

Validation is an activity that has become a greatest concern across the global healthcare industry. These are the critical steps for qualification,

User requirement specification (URS):

It is the basic requirement for the procurement of Isolator.

The following activities are defined for a batch process, and therefore appropriate adaptation is necessary when they are applied to intermittent or continuous sterilization processes.

A. Equipment qualification—The equipment qualification for vapor sterilization mimics that of other sterilization processes in order to confirm that the equipment has been properly installed and operates as intended.
B. Empty chamber parameter distribution—Although multipoint measurement is possible, it lacks correlation to surface microbial kill. Humidity and temperature measurements, along with chemical indicators, can provide a limited indication of sterilant distribution. BIs are not required in the evaluation of the empty chamber.
C. Component mapping—For surface sterilization, internal mapping of load items is not required. Although vapors are primarily used as surface sterilants, when they are used for packaged articles with internal surfaces and volumes that need to be sterilized, internal mapping should be performed, with BIs placed in difficult-to-penetrate locations to confirm process lethality.
D. Load mapping—Humidity and temperature measurements, along with chemical indicators, can provide a limited indication of sterilant distribution on component surfaces. BIs are not required. Effects of load size and patterns should be assessed.
E. Biological indicators—The use of multiple BIs at each test location is recommended to more adequately support the process lethality.
F. Process confirmation and microbiological challenge—The core of the validation activity is the confirmation of acceptable process parameters and inactivation of the microbial challenge. Proof of cycle efficacy is provided in replicate studies in which the BIs are killed and chemical or physical measurements are utilized

The essential practices required to maintain validated status include calibration, physical measurements, use of BIs, physical or chemical integrators and indicators, ongoing process control, change control, preventive maintenance, periodic reassessment, and training.

[9]Halvorson-Ziegler equation

The reason to use multiple BIs at each test location is because we need each BI to be a statistical replicate of its neighbor. I understand there may be reader skepticism at this point as we have a manufacturer of BIs endorsing a policy that seemingly increases BI consumption three-fold. However, this does not need to be the case. Instead of monitoring 100 discrete locations throughout the isolator, the validation technician can identify the 30 or 40 most difficult to sterilize locations and use triplicate BIs at each of these challenge locations, thus consuming 90 or 120 BIs per cycle. With statistical replicates in place, we now have the ability to use the Halvorson-Ziegler equation4

to calculate the most probable number (MPN) of surviving organisms IF we observe a case where one (or two) of the three replicate BIs test growth-positive. When only one BI is used at each test location, and that one BI yields a growth-positive result, there is no way to calculate if that result was due to one surviving spore, or hundreds-of-thousands of surviving spores. The Halvorson-Ziegler equation is:

MPN = ln (n/r)

where:

MPN = Most Probable Number of surviving spores

ln = natural log function

n = number of replicate BIs at each discrete test location

r = number of growth-negative BIs at each discrete test location

Let us look at an example of how we would proceed if in a VHP cycle we observed one positive and two negative BIs (i.e. n = 3 and r = 2) at a particular location where triplicate BIs were used.

MPN = ln (3/2) = 0.405

What this MPN number indicates is that on average we have 0.405 surviving spores per BI. Two of the three BIs were growth-negative and thus we know that there were zero surviving spores on each of those two BIs. Now that we've calculated the MPN value, the next step is to use this number to calculate the spore log reduction (SLR) associated with this observation of two negative BIs and one positive BI. For this we have the following equation:

SLR = Log10 No – Log10 (MPN)

where:

SLR = spore log reduction

No = the initial spore population on the non-exposed BI

If the Certificate of Analysis for the lot of BIs used indicated an initial spore population of 1.6 x 10^6 spores per BI, the SLR calculation would be:

SLR = Log10 (1.6 x 10^6)– Log10 (0.405)

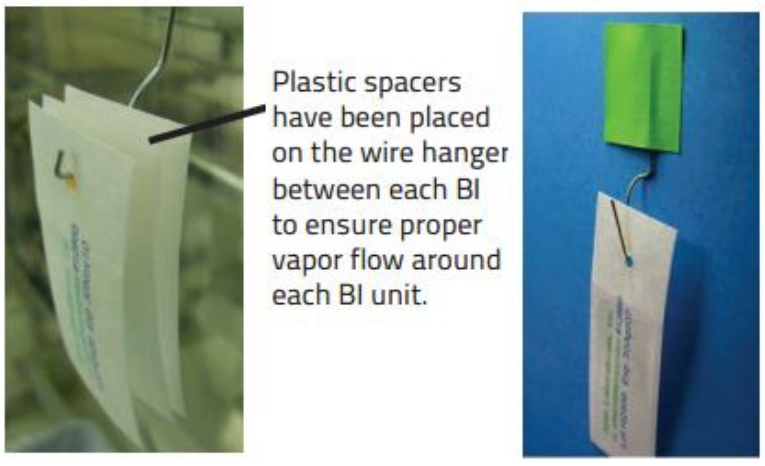
SLR = 6.204 – (-0.393)

SLR = 6.597

Thus, one can see that despite the one growth-positive BI at the location of the triplicate BIs, one can still document that a 6+spore log reduction was achieved at that particular test location. Do be advised that this calculation is ONLY possible when replicate BIs are used. If one were to distribute 100 BIs at 100 discrete test locations, it would not be appropriate to perform the above MPN & SLR calculation as these 100 individual BIs are not replicates of the others.

The next question one might be tempted to ask is, Was that growth-positive BI due to an imperfection in the presentation

of the spores…or was it due to a slight deviation in cycle lethality at that particular test location? Without intending to sound flippant, I suggest the answer to that particular question is, Does it matter? After all, we do have growth-negative observations at that location and the mathematical analysis demonstrates that a 6+ SLR was achieved. Granted, if all three of the replicates are growth-positive, or if one is routinely observing multiple positive BIs at multiple locations over many days of testing, then we must consider that there is a true process deficiency in need of attention. But on the contrary, if one is using the triplicate BI approach and is regularly seeing zero positive BIs at all test locations and an unexpected positive does appear, the above analysis should allow the cycle to be passed, regardless of the true cause of the growth-positive result.



[10]**Figure:** Triplicate and single expose of BI demonstration

9. Definitions/Glossary

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.

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.

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.

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.

Critical zone

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

10. FAQ related Isolator

Determine:

• Model of isolator or barrier, and materials of construction

• Type of isolator (open or closed)?

• Airflow (turbulent, unidirectional)?

• Classification of surrounding room environment

• Number and location of gloves or half-suits

• Attire worn by operators (e.g., are sterile gloves worn under isolator gloves?)

• Operating parameters (pressures, air velocities, temperature, humidity)

Is a written maintenance program in place which requires routine documented checks or tests of gloves, half-suits, door seals, etc. for integrity? What type of tests/checks are done and how frequently?

Do written procedures specify glove replacement frequency? If so, what is the frequency and is the SOP followed?

Does the isolator maintain continuous positive pressure and at sufficient levels?

How are materials transferred into and out of the isolator? How robust are the transfer mechanisms?

Are the equipment and surfaces that have direct contact with sterile products and components sterilized by heat. Does it achieve a minimum of a six-log reduction of the BI spores?

What method is used to decontaminate the inner surface of the isolator barrier (e.g., vapor hydrogen peroxide, steam hydrogen peroxide, chlorine dioxide, etc.)? Determine decontamination parameters.

Did the surface decontamination validation study sufficiently address the ability of the sterilant to disperse throughout the chamber and reach all surfaces? Did the decontamination process include the use of Chemical Indicators (CI) to determine the presence or absence of the VHP on the work surfaces and/or the worst case locations to decontaminate? The CI can assists in the evaluation process by providing useful qualitative data. Were replicate BIs placed throughout the isolator, including the most difficult to reach locations (e.g., underneath any items remaining in isolator during sterilization). Are the most difficult to sterilize materials evaluated?

What is the isolator decontamination frequency and is it justified by validation data?

Is a decontamination cycle performed after a power failure or pressure reversal or other unanticipated breach of system integrity?

How often is the isolator decontamination cycle revalidated?

Does the written environmental monitoring program include routine tests for nonviable particles, as well as an appropriate number of microbial tests (e.g., active air and surface samples; gloves samples) during each campaign? Evaluate the tests performed and the testing frequencies.

11. Reference

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