⟨1208⟩ STERILITY TESTING—VALIDATION OF ISOLATOR SYSTEMS

This chapter provides guidelines for the validation of isolator systems for use in sterility testing of compendial articles. [NOTE—In the context of this chapter, "decontaminated" refers to an item or surface that has been subjected to a process that eliminates viable bioburden.]

Isolators—devices that create controlled environments in which to conduct Pharmacopeial sterility tests—have been used since the mid-1980s. An isolator is supplied with air through a HEPA or better air filter and is able to be reproducibly decontaminated. Closed isolators, which are systems with no direct opening to the external environment, are normally used for sterility testing, although open isolators which allow the egress of materials through a defined opening that precludes the entry of contamination by means of air overpressure may be used. Closed isolators use only decontaminated interfaces or a rapid-transfer port for the transfer of materials. Isolators are constructed of flexible plastics (such as polyvinyl chloride), rigid plastics, glass, or stainless steel.

Isolator systems protect the test article and supplies from contamination during handling by essentially eliminating direct contact between the analyst and the test articles. All transfers of material into and out of the isolator are accomplished in an aseptic fashion while maintaining complete environmental separation. Aseptic manipulations within the isolator are made with half-suits, which are flexible components of the isolator wall that allow the operator a full range of motion within the isolator, or by gloves and sleeves. Operators are not required to wear special clean-room clothing for conducting sterility tests within isolators; standard laboratory clothing is adequate, although a pair of sterile gloves is frequently worn under the isolator gloves as an added precaution against contamination entering the isolator enclosure and for hygiene purposes. The interior of the isolator is treated with sporicidal chemicals that result in the elimination of all viable bioburden on exposed surfaces.

ISOLATOR DESIGN AND CONSTRUCTION

Air Handling Systems

An isolator used for sterility testing is equipped with microbial retentive filters (HEPA filters or better are required). At rest, the isolator meets the particulate air-quality requirements for an ISO Class 5 area as defined in ISO 14644-1 through -3* (see *Microbiological Control and Monitoring of Aseptic Processing Environments* ⟨1116⟩). However, the isolator need not meet Class 5 conditions during an operation that may generate particulates, and no requirements for air velocity or air exchange rate exist. The isolator should be sealed well enough during decontamination that the dissemination of sporicidal vapors or gases into the surrounding environment is kept to appropriately low levels. When direct openings to the outside environment exist, constant air overpressure conditions maintain sterile conditions within the isolator. In general, both open and closed isolators are maintained at positive pressure relative to the surrounding environment, and overpressures of 20 Pa or more are typical. The user should never exceed the maximum pressure recommended by the isolator manufacturer. Airflow within isolators used for sterility testing is either unidirectional or turbulent.

Transfer Ports and Doors

Isolators may be attached to a "pass-through" decontaminator or transfer isolator to enable the direct transfer of sterile media, sterile dilution fluids, and sterile supplies from the decontaminator into the isolator system. Rapid transfer ports (RTPs) enable two isolators, i.e., the work station and transfer isolator, to be connected to one another, so that supplies can be moved aseptically from one isolator to another. Aseptic connections between two isolators or an isolator and an RTP-equipped container can be made in unclassified environments using RTPs. The nonsterile surfaces of the RTP are connected using locking rings or flanges. A compressed gasket assembly provides an airtight seal, thereby preventing the ingress of microorganisms.

When the two RTP flanges are linked to form an airtight passage, a narrow band of gasket remains that could harbor microbial contamination. This exposed gasket should be routinely disinfected immediately after the connection is made, and before materials are transferred through the RTP. Good aseptic technique is used when transferring materials and care is taken not to touch the gasket with the materials being transferred or with the gloved hands.

Preventive maintenance and lubrication of the gasket assemblies on the flanges is performed according to the RTP manufacturer's recommendations. The RTP gaskets are changed at the recommended frequency and periodically checked for damage, because cut or torn gaskets cannot make a truly airtight seal.

Selection of a Location for the Isolator

Isolators for sterility testing need not be installed in a classified clean room, but it is important to place the isolator in an area that provides limited access to nonessential staff. The appropriate location provides adequate space around the isolator for moving transfer isolators, staging of materials, and general maintenance. No environmental monitoring of the surrounding room is required.

Temperature and humidity control in the room is important to operator safety and comfort and is critical for the effective utilization of certain decontamination technologies. Uniform temperature conditions in the room are desirable when temperature-sensitive decontamination methods are employed. Care should be taken in locating the isolator so that cold spots are avoided that might result in excessive condensation when condensing vapors are used for decontamination.

* International Organization for Standardization (ISO) International Standards 14644-1, -2, -3, and -7

VALIDATION OF THE ISOLATOR SYSTEM

The isolator system must be validated before its use in sterility testing as part of a batch release procedure. To verify that the isolator system and all associated equipment are suitable for sterility tests, validation studies are performed in three phases: installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). The following sections contain points to consider in the validation of isolator systems for sterility testing. The assignment of test functions to a particular phase of the validation program (i.e., IQ, OQ, and PQ) is not critical, as long as proper function of the isolator is demonstrated and documented before its use in compendial *Assays.*

Installation Qualification (IQ)

The IQ phase includes a detailed description of the physical aspects of the system, such as the dimensions, internal configuration, and materials of construction. The unit layout is diagrammed with interfaces and transfer systems clearly and dimensionally indicated. Compliance with design specifications for utility services, such as air supply, vacuum, external exhaust, and temperature and humidity control, is verified. Other equipment used with the isolator system is also described in detail; if any revisions to design specifications are made, these are included. Equipment manuals and copies are catalogued and stored where they can be retrieved and reviewed. Compliance of drawings to design specifications is verified. All drawings and process and instrumentation diagrams are catalogued, stored, and are retrievable.

All documentation is reviewed to verify that it precisely reflects the key attributes of the installed system. This establishes a general benchmark for the isolator system's compliance with design specifications and installation requirements.

Potential process-control or equipment problems that could cause system failure during operation are identified and documented during failure-mode analysis and hazard analysis. The system is modified, if necessary, to minimize the risk of failure, and critical control point methods are established.

The results of the IQ are summarized in an Installation Qualification Report. The following documentation is suggested.

EQUIPMENT

The equipment is listed with its relevant design specifications. The IQ verifies that equipment meeting the appropriate design specifications was received and that it was installed according to the manufacturer's requirements.

CONSTRUCTION MATERIALS

The construction materials of critical system components are checked for compliance with design specifications. The compatibility of the intended decontamination method with the construction materials is verified.

INSTRUMENTS

System instruments are listed with their calibration status.

UTILITY SPECIFICATIONS

All utilities required for operation—as defined in the operating manuals and process and instrumentation diagrams—are checked for availability and compliance with design specifications. Any connection between utility systems and the isolator system is inspected and conformance of these connections to specifications is verified.

FILTER CERTIFICATION

HEPA filters and other microbial retentive filters are tested and certified; copies of test results and certificates are included in the IQ summary. Purchase orders are reviewed and conformance of the air filtration system to specifications is verified.

COMPUTER SOFTWARE

All computer software associated with the isolator system is listed with its name, size, and file revision number. The master computer disks are checked for proper labeling and stored securely.

Operational Qualification (OQ)

The OQ phase verifies that the isolator system operates in conformance to functional specifications.

OPERATIONAL PERFORMANCE CHECK

This test verifies that all alert and alarm functions comply with their functional specifications. The system's ability to comply with all set points and adjustable parameters is verified.

ISOLATOR INTEGRITY CHECK

The integrity of the isolator is maintained during all normal operating conditions. A leak test is performed to verify the compliance with the manufacturer's functional specifications and to ensure safety prior to charging the isolator with a

decontaminating sporicidal chemical. To safeguard against adventitious contamination, isolators are operated at a suitable positive pressure during normal operation. Validation studies must show that the air pressure set point can be maintained and controlled during operation.

DECONTAMINATION CYCLE VERIFICATION

A decontamination cycle that is the function of the decontamination equipment in concert with the isolator(s) is verified.

Different decontamination methods can be used to eliminate bioburden from isolator systems and supplies. Among the chemicals that have been used to treat isolators are peracetic acid, chlorine dioxide, ozone, and hydrogen peroxide; each has different requirements for exposure conditions and process control. It is critical to comply with the manufacturer's operational requirements for the selected decontamination method and to describe them in the functional specifications. The temperature inside the isolator is also important, particularly for hydrogen peroxide vapor decontamination, where it is critical to maintain the concentration relative to the condensation point. Some sterilization chemicals, such as chlorine dioxide and ozone, require the addition of moisture to the isolator prior to decontamination. When elevated relative humidity is required, the ability to control it must be verified during OQ.

It is also important to verify the concentration and distribution of the decontaminating chemical. When applied in gaseous or vapor form, the distribution may be evaluated using chemical indicators, spectroscopic methods, or electronic sensors.

Gas and vapor decontamination methods may require fans in the isolator to distribute the chemical evenly. The location and orientation of these fans are adjusted to ensure optimum air distribution. If the isolator utilizes a recirculating unidirectional airflow system, distribution fans may not be required, but this should be evaluated on a case-by-case basis. Because shelving units, equipment, glove-and-sleeve assemblies, and half-suits have an impact on distribution patterns, distribution checks are done with the isolator fully loaded with equipment and supplies, and the setup of these units is defined and documented.

Many installations use smaller transfer isolators as portable surface decontamination units. In these transfer isolators, test articles and supplies are treated chemically to eliminate bioburden before transfer through an RTP into the testing isolator. Its loading configuration is defined, and configuration drawings are reviewed and verified during the OQ. [NOTE—The decontaminating chemicals used in isolators work on the surfaces of materials; therefore, any surface that is occluded will not be treated and could contain viable bioburden. Special precautions should be in place for treating surfaces known to be occluded with a sporicide if such surfaces may be revealed during the conduct of sterility tests.]

Decontamination agents need to be removed from the isolator after the exposure period, which is accomplished by a current of fresh air provided either by the decontamination equipment or by utilizing the isolator air handling system. Aeration is accomplished either in an open loop, in which the gas is exhausted through a vent to the atmosphere, or in a closed loop, in which the chemical is removed and destroyed by the decontamination equipment. The aeration system is checked; if an open-loop configuration is used, the external exhaust system's flow and safety are checked.

DECONTAMINATION CYCLE DEVELOPMENT

When the OQ is completed, decontamination cycle development is performed to establish the parameters necessary for process control during routine decontamination cycles. Any of the methods generally used in the industry for the validation of decontamination processes—including bioburden-based, fractional cycle, and overkill methods—are adequate. The decontamination process is challenged with biological indicators (BIs). The spore population and resistance of the BIs to the decontamination conditions being applied are known. Wherever possible, a D value estimate is done for each B1 system or, alternatively, a survivor curve for the BI system is obtained (see *Biological Indicators—Resistance Performance Tests* ⟨55⟩); it is acceptable to obtain the D value from the BI vendor.

Performance Qualifications (PQ)

The PQ phase verifies that the system is functioning in compliance with its operator requirement specifications. At the completion of the PQ phase, the efficacy of the decontamination cycle and, if appropriate, the adequacy of decontaminating chemical venting are verified. All PQ data are adequately summarized, reviewed, and archived.

CLEANING VERIFICATION

In general, cleaning is not critical for sterility testing applications. However, residual products are a concern in multiproduct testing, particularly for aggressive antimicrobial agents, because these materials could interfere with the ability of subsequent tests to detect low levels of contamination in the product. Concerns about contamination with the product are heightened when it is an inherently antimicrobial powder, because powders are more readily disseminated. Cleaning to a level at which no visible contamination is present is adequate for sterility test isolator systems and is a suitable operator requirement specification. The cleaning method, frequency, equipment, and materials used to clean the isolator are documented.

DECONTAMINATION VALIDATION

The interior surfaces of the isolator, the equipment within the isolator, and the materials brought into the isolator are treated to eliminate all bioburden. The decontamination methods used to treat isolators, test articles, and sterility testing supplies are capable of reproducibly yielding greater than a three-log reduction against highly resistant biological indicators (see *Biological Indicators for Sterilization* ⟨1229.5⟩), as verified by the fraction negative or total kill analysis methods. Total kill analysis studies are suitable for BIs with a population of 10^3 spores per unit, while fraction negative studies are suitable for BIs with a population of 10^5 or greater. A sufficient number of BIs are used to prove statistical reproducibility and adequate distribution of the decontaminating agent. Particular attention is given to areas that pose problems relative to the concentration of the agent. A

larger number of BIs may be required in isolators that are heavily loaded with equipment and materials. The ability of the process to reproducibly deliver a greater than three-log kill is confirmed in three consecutive validation studies.

The operator establishes a frequency for re-decontamination of the isolator. The frequency may be as short as a few days or as long as several weeks, depending on the sterility maintenance effort (see *Maintenance of Asepsis within the Isolator Environment*).

PACKAGE INTEGRITY VERIFICATION

Some materials are adversely affected by decontaminating agents, which can result in inhibition of microbial growth. Of concern are the penetration of decontaminating agents into product containers; accessory supplies such as filter sets and tubing; or any material that could come in contact with product, media, or dilution fluids used in the sterility test. It is the responsibility of the operator to verify that containers, media, and supplies are unaffected by the decontamination process. Screw-capped tubes, bottles, or vials sealed with rubber stoppers and crimp overseals have proven very resistant to the penetration of commonly used decontaminating agents. Wrapping materials in metal foil or placing them in a sealed container will prevent contact with the decontaminating agent; however, these procedures may also result in some surfaces not being decontaminated. In some cases, the use of shorter duration decontamination cycles and reduced concentrations may be necessary to minimize penetration of decontaminating agents into the package or container. Cycles that provide a less than three-log kill of resistant BIs may be acceptable provided microbiological analysis of the environment proves that the isolator(s) are free of recoverable bioburden.

In many cases, the operator will choose to treat the surfaces of product containers under test with the decontaminating agent in order to minimize the likelihood of bioburden entering the isolator. It is the responsibility of the operator to demonstrate, via validation studies, that exposure of product containers to the decontaminating agent does not adversely affect the ability of the sterility test to detect low levels of contamination within these test articles. It is suggested that the ability of the package to resist contamination be examined using both chemical and microbiological test procedures. Bacteriostasis and fungistasis validation tests must be performed using actual test articles that have been exposed to all phases of the decontamination process (see *Sterility Tests* ⟨71⟩). This applies to medicinal device packages as well as pharmaceutical container and closure systems.

Validation studies determine whether both sterility test media and environmental control media meet the requirements for *Growth Promotion Test of Aerobes, Anaerobes, and Fungi* under *Sterility Tests* ⟨71⟩.

MAINTENANCE OF ASEPSIS WITHIN THE ISOLATOR ENVIRONMENT

The ability of the isolator system to maintain an aseptic environment throughout the defined operational period must be validated. In addition, a microbiological monitoring program must be implemented to detect malfunctions of the isolator system or the presence of adventitious contamination within the isolator. Microbiological monitoring usually involves a routine sampling program, which may include, for instance, sampling following decontamination on the first day of operation and sampling on the last day of the projected maintenance of asepsis period. Periodic sampling throughout the use period can be performed to demonstrate maintenance of asepsis within the isolator.

The surfaces within the isolator can be monitored using either contact plates for flat surfaces or swabs for irregular surfaces. However, because media residues could impose a risk on isolator asepsis, these tests are generally best done at the end of the test period. If performed concurrently with testing, care is used to ensure that any residual medium is removed from isolator surfaces, and that those surfaces are carefully cleaned and disinfected. Active air samples and settling plates may be used, but they may not be sufficiently sensitive to detect the very low levels of contamination present within the isolator enclosure.

A potential route for contamination to enter the isolator is during the introduction of supplies and samples into the enclosure. Validating that all materials taken into the isolator enclosure are free of microbial contamination is critical, as is periodic inspection of gaskets to detect imperfections that could allow ingress of microorganisms. Gloves and half-suit assemblies are another potential source of microbial contamination. Gloves are of particular concern because they are used to handle both sterility testing materials and test articles. Resistance to puncture and abrasion should be considered in the selection of gloves and sleeves. Hypalon materials are resistant to both chemical sporicides used in the decontamination of isolators and to punctures and are available in several thicknesses to provide adequate tactile feel through the gloves while maintaining their integrity.

Very small leaks in gloves are difficult to detect until the glove is stretched during use. There are several commercially available glove leak detectors; the operator ensures that the detectors test the glove under conditions as close as possible to actual use conditions. Microbiological tests are used to supplement or substitute physical tests. [NOTE—Standard "finger dab plates" may not be sensitive enough to detect low levels of contamination. Submersion of the gloves in 0.1% peptone water followed by filtration of the diluent and plating on growth media can detect loss of integrity in the gloves that would otherwise go unnoticed.]

Continuous nonviable particulate monitoring within the isolator's enclosure is ideal, because it can quickly detect filter failure. A second choice is periodic monitoring using a portable particle counter. Sampling for particles must be done in a manner that poses no risk to the maintenance of asepsis within the isolator.

INTERPRETATION OF STERILITY TEST RESULTS

A sterility test resulting in a false positive in a properly functioning and validated isolator is very unlikely if bioburden is eliminated from the isolator interior with a high degree of assurance; if gloves, sleeves, and half-suits are free of leaks; and if the RTPs are functioning properly. Nevertheless, isolators are mechanical devices and good aseptic techniques are still required. A

decision to invalidate a false positive is made only after fully complying with the requirements of *Observation and Interpretation of Results* under *Sterility Tests* ⟨71⟩.

TRAINING AND SAFETY

As with sterility testing conducted in conventional clean rooms, operators are trained in procedures that are specific to their isolator. Use of proper aseptic techniques is vital to the conduct of sterility tests in isolators, just as it is in clean rooms. Therefore, training in proper aseptic techniques is required for all sterility testing technicians. All training sessions and the evaluation of the operator's performance are documented in the individual's training record. Training of all personnel in the appropriate safety procedures necessary for the operation and maintenance of the isolation system is imperative.

Personnel safety in the use of a decontaminating agent must be assessed. Material Safety Data Sheets, or equivalent documents, are available in the immediate area where the decontaminating agent is being used. All storage and safety precautions are followed. An operational readiness inspection of the safety of the isolator and all associated equipment is performed and documented prior to placing the unit in service.