# Validation Strategy for a Sterility Testing Isolator Project

James R. Rickloff, Partner/Scientific Director
Advanced Barrier Concepts, Inc.

This document provides an overview on how to properly validate sterility test isolator systems and then, via a case study, describes a strategy for streamlining the validation process when equivalent isolators and/or generators are installed in a sterility test suite.

In general, the validation work for a sterility test isolator project includes all of the major steps outlined in the following block diagram summary:

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| 1. Facility Qualification | Key facility attributes include:<br>a. HVAC/temperature control<br>b. safety monitoring<br>c. controlled access |
| 2. Equipment Qualification | Standard IQ/OQ of isolator and sterilant generator installation and functional testing, including alarms testing. Also, the exhaust system is verified as an extension of the isolator. |
| 3. BI D-value Determination | Provides a baseline resistance under specific conditions for the BIs used for testing. This baseline value can be compared to subsequent BI lots (used in revalidation studies) to verify that a reasonably equivalent challenge is being utilized. |
| 4. Decontamination Cycle Development (TCs, CIs, & BIs) | Cycle parameters for the VHP1000 are established, initially based upon computer models using the temperature ranges established. Parameters are then verified in PQ. Consistent temperature and gas distribution provides optimal performance. |
| 5. Sporicidal Efficacy Qualification | A minimal (fractional) cycle is validated in triplicate with the intention of providing a total kill of all $10^6$ BIs (*Geobacillus stearothermophilus* spores). |
| 6. Aeration Qualification | The normal cycle (typically, the validated exposure time is increased by 20% to account for potential system variability) is challenged in triplicate to verify removal of the sterilant residues that may interfere with the sterility test. |
| 7. Sterilant Ingress Testing | Product containers filled with distilled water are exposed to back-to-back decontamination cycles and then chemically assayed to verify no sterilant penetration. |
| 8. Residue Effects Testing (Process Simulation to rule out false negatives) | Typically select 3-6 organisms (10-100 CFU) based upon USP growth promotion organisms and lab history (key environmental isolates) to provide evidence that the sterilant does not interfere with the sterility test and environmental monitoring techniques. |

The goal is to first qualify the room and equipment contained within it for mechanical and electrical operation, including HVAC characteristics. Following equipment qualification, the decontamination process for bioburden reduction of test materials (including the exterior of sample containers) need to be qualified to provide evidence that sufficient kill is achieved in order to provide a germ-free environment for sterility testing. Additionally, aeration times and conditions are established, verifying the removal of the residual sterilant upon completion of the decontamination process. The final phase of testing involves verification that the decontamination process does not interfere with the results of the sterility test on the target product sample. This is achieved by subjecting the testing materials (including the sample containers and sterility testing supplies) to the sterilant (back-to-back decontamination cycles), then demonstrating that low levels of microbial contamination can still be detected with the sterility test.

This procedure is termed 'false-negative' testing and it is a form of a process simulation that provides evidence that the decontamination process does not interfere with the sterility test or environmental monitoring results.

**Case Study – Small Scale Testing**

A vaccine manufacturer provided Walker Barrier Systems (New Lisbon, WI) with a set of requirements for testing a single lot of final product containers (vials) and bulk samples (Hyclone™ bags) on a daily basis in a fully automated isolator system that would both require the use of Millipore Steritest kits for sample recovery. At maximum loading, the total decontamination cycle time including aeration would need to be <5 hours to permit time for the testing within a single 8-hour shift. The end-user decided to purchase two (2) identical turbulent flow isolator systems shown in the diagram below to handle current and anticipated test requirements for the facility and to provide for redundancy in the event that one (1) system was down for maintenance, etc. Two (2) STERIS VHP®1000ED-AB Generators were purchased for the decontamination of the enclosures using hydrogen peroxide ($H_2O_2$) gas.

Four Glove Sterility Testing Isolator

Initially, a robust decontamination cycle was developed for the maximum final product load configuration in Sterility Test Isolator #1 using VHP1000ED-AB Generator #1. The sterilant gas concentration was based upon the minimum surface temperature within the enclosure at the end of the Dehumidify Phase (20°C). It was determined that a 30-minute exposure was sufficient to inactivate all of the BIs placed throughout the enclosure and load. The developed 'fractional' cycle was then validated in triplicate for the maximum final product and bulk sample load configurations, which was successful.

Aeration studies using a 'normal' exposure time of 36 minutes (20% longer than the validated time listed above) were performed in triplicate on both maximum load configurations. Three (3) hours of isolator exhaust (pressure set at 1.0" of water column) were required to consistently reduce the $H_2O_2$ gas concentration to at or below 0.5 ppm for each load configuration. The isolator exhaust used a 'thimble' connection that allowed isolator air to be diluted into a room exhaust air line, which eliminated the concern of pressure drop on reducing airflow rate if a hard connection was made to the isolator's exhaust pipe.

The next goal of the validation effort was to verify that temperature distribution and sporicidal efficacy of $H_2O_2$ gas during the decontamination of Sterility Test Isolator #1 was equivalent to the decontamination of Sterility Test Isolator #2 for each validated load configuration. Temperature distribution and sporicidal efficacy tests were also performed using VHP1000ED-AB Generator #2 attached to Sterility Test Isolator #1 to demonstrate generator equivalency for each validated load configuration.

A single TC/BI study was performed on each load configuration to demonstrate isolator and generator equivalency and each of the tests were successful.

The aeration of Sterility Test Isolator #2 to $H_2O_2$ gas levels considered acceptable for sterility testing and for ensuring operator safety ($\leq$0.5 ppm) was then shown to be equivalent to the aeration time validated for Sterility Test Isolator #1 for each validated load configuration. Finally, a single test verified that VHP1000ED-AB Generator #2 was equivalent to VHP1000ED-AB #1 in terms of aerating Sterility Test Isolator #1 to $\leq$21 ppm of $H_2O_2$ gas for each validated load configuration after exposure to the 'normal' exposure time of 36 minutes.

In conclusion, a 4-hour, 14-minute "robust" decontamination cycle was validated for the sterility test isolator system in this facility, which met the user requirement of <5 hours. The validation data supported the use of either isolator connected to either generator for the decontamination and sterility testing of either final product or bulk samples using the same cycle settings; thus providing the end-user with sufficient operational flexibility in the event that some of the equipment was out of service. Process simulation studies were also successful in verifying that false negative sterility test or environmental monitoring results were not occurring even following back-to-back decontamination cycles. This data supported the use of product samples or supplies that were exposed to more than one (1) decontamination cycle in the event that the previous cycle was aborted for some reason.

Walker Barrier Systems is pleased to announce our partnership agreement with Advanced Barrier Concepts, Inc. Advanced Barrier Concepts was founded in 1994 and currently has offices in Cary, North Carolina and Gieres France to provide scientific and technical services that are dedicated to the advancement of state-of-the art isolation, aseptic processing, and sterilization systems technologies to the pharmaceutical, biotechnology, and medical device industries.

The Advanced Barrier Concepts team is led by James Rickloff and William Little, both of whom have had prior industry experience in the development of the vaporized hydrogen peroxide sterilization technology that is widely used for the decontamination of isolator systems. In partnership with Walker Barrier Systems, Advanced Barrier Concepts personnel will provide validation services on Walker Barrier Systems isolators and on the chosen decontamination system generator. These services can include standard or custom GAMP® 5 documentation and IQ/OQ/PQ protocols along with the execution of the protocols, including advanced decontamination cycle development and validation. Biological Indicator D-value studies, sterilant intrusion and false negative testing, and custom SOP development are also available to Walker Barrier Systems clients. Finally, hands-on teaching of the entire system can be provided to give operators the confidence they need to embrace this new technology on a daily basis. Walker Barrier Systems is dedicated to providing high quality isolators to our customers and by partnering with Advanced Barrier Concepts can offer companies a single source for complete system design, manufacture, and validation of your advanced aseptic process.