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Isolators Selection, Design, Decontamination, and Validation

by Nick Barbu and Robert Zwick

This article presents the selection, design, and validation of isolators to be used by the Musculoskeletal Transplant Foundation for the production of Demineralized Bone Matrix putty.

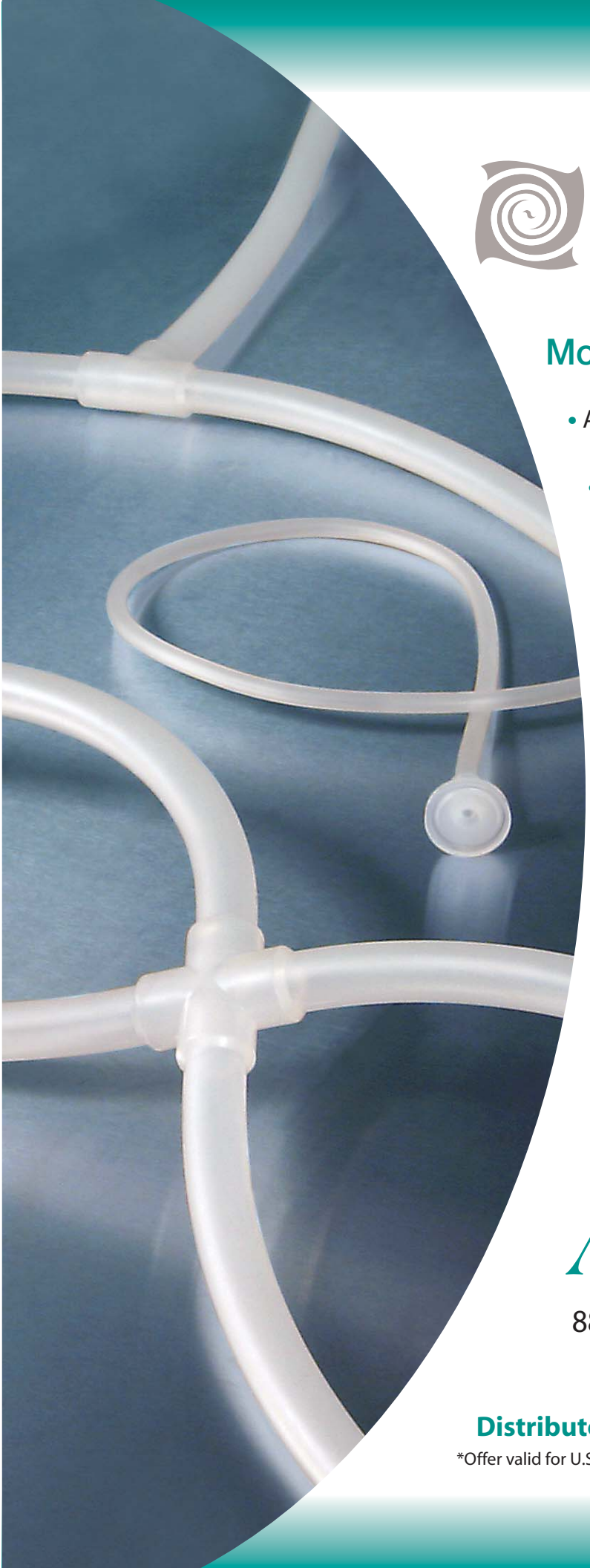
MTF is a supplier of Allograft – bone and dermal tissue. The purpose of this isolator project involves the production of Demineralized Bone Matrix (DBX) putty. DBX putty is processed human bone that has been demineralized and combined with sodium hyaluronate (HY), which is a naturally derived material not of animal origin that is both biocompatible and biodegradable. The combination of demineralized bone and sodium hyaluronate results in a putty-like consistency for ease and flexibility of use during surgical application. DBX putty is intended for use as a demineralized bone matrix for voids or gaps that are not intrinsic to the stability of the bony structure. DBX putty is indicated for treatment of surgically created osseous defects or osseous defects created from traumatic injury. DBX putty can be used as follows:

- Extremities
- Posterolateral spine
- Pelvis
- Ridge augmentation
- Filling of extraction sites
- Cranium
- Craniofacial augmentation
- Mandibular reconstruction
- Repair of traumatic defects of the alveolar ridge, excluding maxillary and mandibular fracture
- Filling resection defects in benign cysts, or other osseous defects in the alveolar ridge wall
- Filling of cystic defect
- Filling of lesions or periodontal origin
- Filling of defects of endodontic origin

DBX putty is packaged in a glass syringe and must be extruded into a sterile basin and is not injected directly into the operative site. DBX putty can be used alone or mixed with autogenous or allograft bone or with bone marrow aspirate. Since the DBX putty is introduced into the body, it must be produced and packaged under aseptic conditions and procedures. Aseptic process refers to the condition of being free from all forms of life, including bacteria, fungi, and viruses. Aseptic technique refers to efforts to maintain a sterile field during a procedure to prevent infection. These efforts include utilizing sterilized instruments and supplies and requiring staff to wear sterile gloves and other clothing, such as caps, gowns, and masks to reduce potential contamination.

The process whether conducted in either a cleanroom, biological safety cabinet, or an isolator is largely the same with the exception of the decontamination cycle. In order to maintain the highest aseptic techniques, it was decided to move the DBX putty process to isolators for their ease of use in cleaning and decontamination.

Barrier isolation technology has been recognized by the Food and Drug Administration (FDA) for a number of years as an effective method of aseptic processing. The DBX mix-fill, measuring and packaging processes were all set to be performed in isolators, decreasing the likelihood of microbial contamination. Isolators also have the added benefit of increased personal safety and comfort for the processing technicians.



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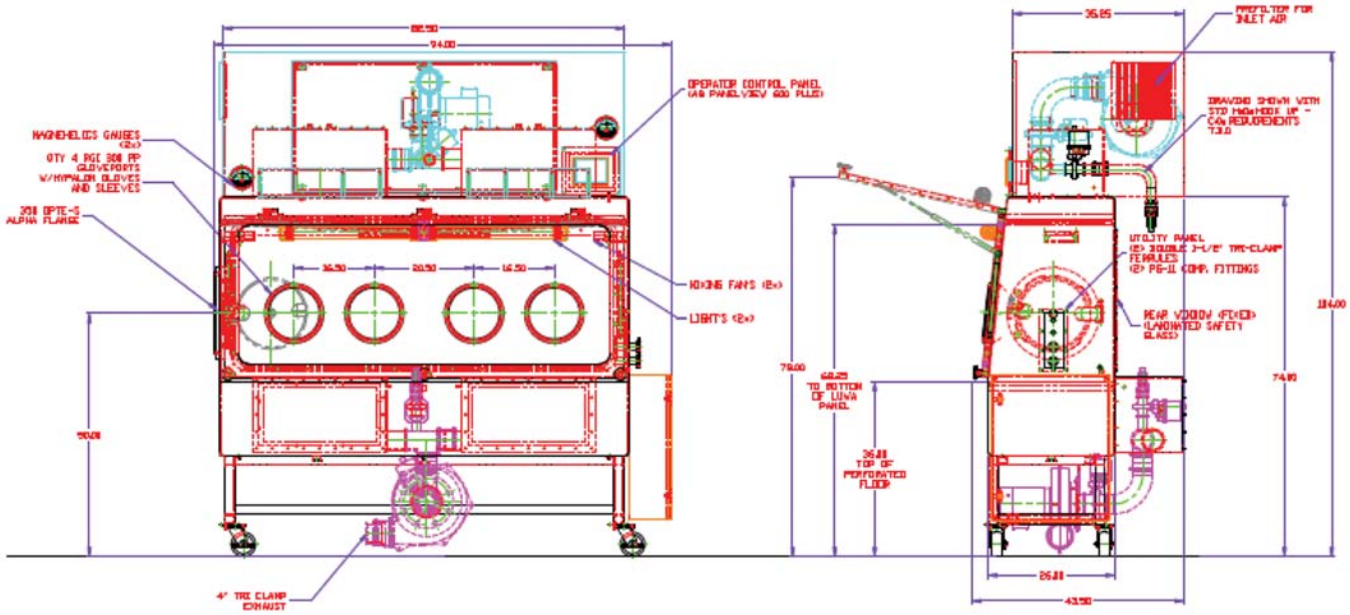

Figure 1. Mix/fill isolator.

Isolator Selection

Isolators technology selection was an important step in the process design with many factors. What size should the isolator be? What equipment should be inside the isolator? Which isolator manufacturer should be selected? After considerable research and discussion an isolator supplier was chosen. The production process was broken down to two isolators: 1. the mix fill isolator for mixing and filling and 2. the packaging isolator where final product packaging is performed. Both the mix fill and packaging isolators are about 100 cu ft (2.8 cu m) each. The packaging isolator was designed to hold the packaging equipment which added a sealer compartment to its layout. The packaging isolator also required the ability to decontaminate and transfer materials in and out of the main chamber which added an air lock to its layout. See Figures 1 and 2.

Decontamination Method Selection

Once the isolator was chosen, the decontamination method was selected. In order for this process to be economically

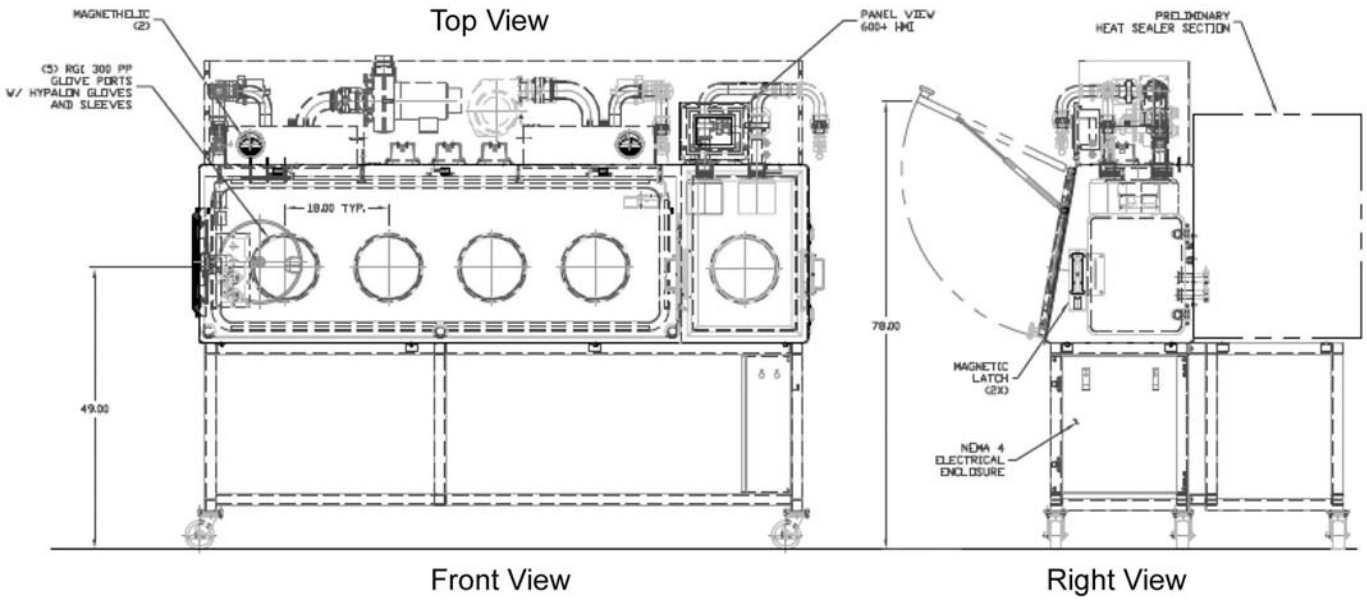

Figure 2. Packaging isolator.

The Musculoskeletal Transplant Foundation (MTF) is the nation's leading tissue bank. One of the ways MTF is raising the standards of production to higher levels has been by investigating, designing, selecting, and validating the use of isolators in the production of Demineralized Bone Matrix (DBX) putty. To accomplish this, the authors had to understand isolation technology and identify the requirements of production, decontamination, and overall validation. Isolators were used in conjunction with chlorine dioxide gas decontamination, working together to provide a simple systems integration. Both products met the needs of MTF for ease of use, design, flexibility, decontamination cycle effectiveness, and time.

feasible, the decontamination process had to take less than two hours. If the sterilization/decontamination time exceeded two hours, the product would not be cost effective enough to warrant the change from cleanroom processing to isolation processing. The choice for sterilization/decontamination method was between vapor phase hydrogen peroxide and chlorine dioxide gas. Both methods were known to be effective, as both are registered with the US-EPA as sterilants. Similarly, Vapor Phase Hydrogen Peroxide (VPHP) and Chlorine Dioxide (CD) gas have both been used in cleanroom environments and in isolators.

VPHP has been around a long time, as it was developed in the late 1970s.[17] It has benefits when used for sterilization, such as it does not leave residues. The vapor is generated by boiling or vaporizing a solution of hydrogen peroxide, typically 35% hydrogen peroxide/65% water. This vapor is then injected into the target chamber. The VPHP process will have varying amounts of condensation since VPHP is not a true gas at room temperatures (hydrogen peroxide boiling point 109°C). The condensation amount is minimized or maximized depending upon whether a high or low RH system is utilized. Another issue with VPHP is a potential for poor distribution[9,21] and penetration abilities into 5 mm gaps[23] and small tubing and openings.[3] The potential for reduced distribution was of particular concern to MTF due to the complex surface geometry of the sealer used in its packaging isolator. For these reasons, MTF also looked at chlorine dioxide gas as a decontamination method.

Chlorine Dioxide (CD) has been used in many applications, such as studies and research,[11,8] isolators,[1,4] processing vessels,[5] HEPA housings with small tubing,[3] BSC's (NSF Standard 49, 2008)[15], rooms,[13,20] and large facilities.[2,14] It is a gas at room temperatures (boiling point 11°C) and is not considered to be carcinogen by IARC, NTP, OSHA, and ACGIH. CD gas does not leave a residue. As a true gas, complex surface geometry is not a factor as the gas will achieve complete, natural distribution of the space it is contained within. Chlorine dioxide has shown promising results with organic loads, including studies at Public Health Agency of Canada showing kill with organic soiled loads,[12] wood, carpet and ceiling tiles,[18] under mouse cage bedding,[22] and HEPA filters loaded with soil.[15]

Chlorine dioxide gas was chosen as the sterilant due to its fast cycle times and evidence of its effectiveness. With the reduced cycle times, MTF's decision to move forward with isolators became feasible. MTF's requirement was to demonstrate a complete kill of 6-log biological indicators with a total cycle time of less than two hours including chamber leak test and aeration. To accomplish this, the Cloridox-GMP chlorine dioxide gas generator was chosen as seen in Figure 3.

An early feasibility study using chlorine dioxide was conducted to test potential concerns about product viability, due to any residual left after decontamination. Tests involved exposing the demineralized bone to concentrations of 5 mg/L for 30 minutes (extreme test) and the demineralized bone and sodium hyaluronate to 0.1 mg/L for 45 minutes to test the maximum residual ClO2 concentration after aeration. The results of these tests gave confidence that aeration down to 0.1 ppm of residual ClO2 (the Permissible Exposure Limit (PEL)) would not affect the efficacy of their final product. The materials were processed into DBX putty and tested for osteoinductivity, pH, penetration, and irrigation. The test results for all samples fell within the requirements for DBX putty.

Residual tests were performed by outside testing laboratories, on final product which had been exposed to post aeration levels of residual ClO2 (0.1 ppm). A method similar to EPA 300.1 and 326.0 using ion chromatography was used to measure oxyhalide disinfection (ClO2) by-products in an extract of DBX putty. The limit of detection was determined

Figure 3. Cloridox-GMP and mix fill isolator.

to be 3 ppm, all samples tested below the limit of detection. This gave confidence that if any residual CD gas was left inside the isolator it would be significantly lower than the allowable levels.

Once chlorine dioxide gas was chosen as the decontamination method, the cycles needed to be developed (decontamination Performance Qualification (PQ)). Chlorine dioxide gas cycles are similar to ethylene oxide such that humidity needs to be added before the gas is introduced. Raising humidity in the isolator is a simple process using a very small commercially available steam generator. The steam generator is filled with Water for Injection (WFI) and a heater heats the water to produce the steam. The Cloridox-GMP has an Relative Humidity (RH) probe which measures the RH in real time and turns on the steamer to add RH if it is below the set point. A decontamination cycle for chlorine dioxide contains 5 steps: 1. Precondition, 2. Condition, 3. Charge, 4. Exposure. and 5. Aeration. The precondition step includes two functions: leak testing the isolator and raising the RH to the desired set point. The isolator performs the leak test, which if successful, releases the interlock allowing the Cloridox-GMP to start the cycle by raising the humidity to the set point of 65%. Once the RH is at 65%, the cycle advances to the condition step, where the RH is maintained for 10 minutes. After condition, the cycle advances to the charge step where the chlorine dioxide gas is introduced to the isolator to reach a concentration of 5 mg/L. The chlorine dioxide gas is generated by the following equation: $Cl2_{(g)} + 2NaClO2_{(s)} = 2ClO2_{(g)} + 2NaCl_{(s)}$. In this process, chlorine gas is passed through solid sodium chlorite cartridges and a pure chlorine dioxide gas is produced with no byproducts introduced into the isolator. The sodium chloride byproduct kept inside the cartridges. When the concentration is verified by the real-time concentration monitor to have met process set point, the cycle advances to the exposure step where the concentration is maintained for 50 minutes. If at any point during the cycle the CD concentration drops below the set point, the Cloridox-GMP automatically stops the exposure timer and adds more CD, when the required concentration is restored the exposure timer is restated. So, if the concentration falls below the set point at any time during the exposure, that time is not accumulated in the exposure time. For example, if the exposure time is 30 minutes and the concentration falls below set point for one minute, the overall exposure time will be 31 minutes. This guarantees an exposure at the desired concentration for the desired length of time, thereby ensuring all cycles are efficacious each and every time. The CD concentration is monitored by a photometer which outputs an absorbance value, which corresponds to a concentration of chlorine dioxide measured in mg per liter. Once the exposure time is completed, the aeration or gas removal step starts. During aeration, CD gas is removed from the chamber by allowing clean air into the chamber and removing CD to an outside exhaust. When gases are removed from chambers, typically half the gas molecules are removed with each air exchange. For example, a 100 ft^3 (2.8 m^3) isolator with 5mg/L CD gas concentration and a 50 cfm exhaust (85 cu m/hr) rate, would need approximately 24 minutes, or roughly 15 air exchanges, to bring the CD concentration in the chamber to 0.1 ppm or below. The 0.1ppm (0.3 milligrams per cubic meter (mg/m(3)) concentration level is the eight hour Time Weighted Average (TWA) for the Permissible Exposure Limit (PEL) of chlorine dioxide.

Validation

Validation is a time consuming endeavor for new products, processes, or new equipment. For this case study, it was both a new process (moving to isolators) and new equipment (new isolators and decontamination equipment). The process used to validate the new equipment and process is as follows:

1. Factory Acceptance Testing (FAT) of isolators
2. FAT chlorine dioxide generator
3. Site Acceptance Testing (SAT)/commissioning of isolators
4. Installation Operational Qualification (IOQ) of isolators
5. Performance qualification/decontamination cycle development
6. Process qualification, unique to process being performed in the isolator/s
7. Validation, media fill/aseptic fill, again unique to process preformed in isolators
8. Risk assessment

1. Factory Acceptance Testing (FAT) of Isolators

This step was essentially an IOQ light conducted at the manufacturer's location. The key points for successful isolators FAT were identified prior to the trip. The key tests were a successful leak test. For this test, the isolator was pressurized and monitored for pressure decay over time. The pressure set point was 1.5" of water column with an allowable drop of 0.2" of water. After the pressure decay test, a smoke test was completed. This demonstrated air flow through the isolator chamber. This was particularly important for processes requiring either laminar flow or low particle counts. Finally, a functional verification of all equipment and operator interfaces, check lights, valves, blowers, etc., work in the appropriate operating mode (Decon, Production, Stand By, etc.)

2. FAT Testing for Decontamination System

The FAT for the decontamination testing was done at the manufacturer's facility with the generator to be purchased. A cycle was tested on a small 17 cu ft isolator supplied by the decontamination equipment manufacturer. A few biologi-

cal indicators were placed inside the isolator and a cycle was run. The cycle that was run had process parameters of 65% RH for 30 minutes of conditioning and 5 mg/L concentration of chlorine dioxide gas for an exposure time of 30 minutes. After the exposure, the BIs were incubated for seven days and checked for growth. No growth was observed. In addition to the efficacy testing, a few alarm functions were tested along with consumable change out. All functions tested performed as required. Additionally, the manufacturer of the decontamination equipment performed and documented a complete FAT which tested the proper wiring and cycle functions to ensure the equipment functions according to specifications.

3. Site Acceptance Testing (SAT)

Upon equipment arrival, SAT/commissioning tests were performed. With the exception of verifying the communication between the CD generator and the isolators, these tests were similar to the FAT with more of an emphasis on making sure everything arrived in working order and functioned according to the manufacturer's tests. Both the FAT and SAT are not generally a part of MTF's validation package and were performed primarily to get a feel for whether or not the equipment, people, and process were ready for validation.

4. Installation Operational Qualification (IOQ)of the Isolators

The first step of performing the IOQ was to ID or document the isolator equipment. Each major component was identified along with supporting documentation. The supporting documents are such things as calibration sheets, filter certifications, operational manuals, system drawings, and standard operating procedures. A few examples of major components to verify would be HEPA filters, power supplies, motors, sensors, valves, and most other parts with a model/serial number on them. After supporting documentation and major components are identified and recorded, software versions are then verified to be correct and current.

The next step is the Operational Qualification (OQ). In this step, the equipment functions or modes are verified. Some of the functional tests conducted were power failure and recovery (does the isolator power up and recover from power loss in the right mode?), pressure control (does the isolator maintain the positive pressure that was required?), pressure alarms (does the isolator alarm if pressure drops or spikes?), automated leak test verification, particle count verification, airflow verification (using smoke to verify airflow and the airflow velocity was measured) and lastly the RTP port and beta container were verified to connect and disconnect.

After the isolator was verified to function, the IOQ of the chlorine dioxide gas generator was started. The steps performed here were similar to the isolator IOQ such that the critical equipment was documented along with verification of the equipment and the supporting documentation: calibration certifications, manuals, and drawings. After the documentation was compiled, the software versions were noted. After the IQ was completed the OQ was conducted by loading consumables, alarm testing of key alarms, testing of communication between the isolator and the chlorine dioxide gas generator, power failure recovery and finally cycle verification.

5. Performance Qualification Decontamination Cycle Development

Once all users were trained on the chlorine dioxide gas generator and the training process was documented, the cycle development for the decontamination cycle could begin. This was started by determining a D-value for the biological indicators (fractional negative method used by Stumbo, Murphy and Cochran) and enumerating the BIs to verify the population with the manufacturer's specification. After the D-value and enumeration, the decontamination cycle development began. BIs were placed at various locations within the isolator chambers as seen Figure 4 and 5.

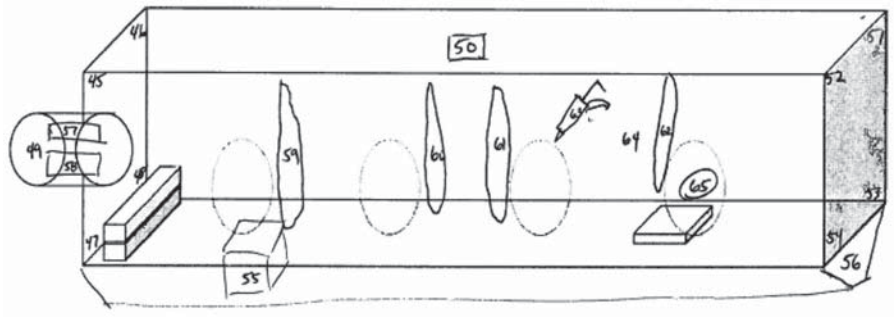

Figure 4. Mix fill isolator BI locations.

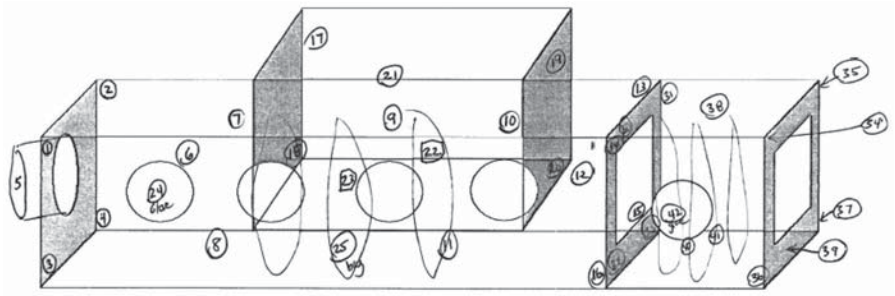

Figure 5. Packaging isolator BI locations.

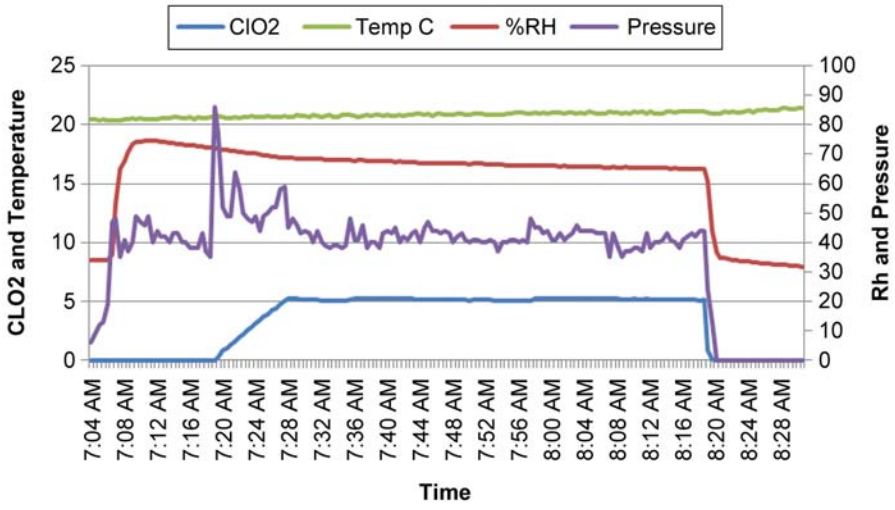

Figure 6. Mix fill isolator decontamination cycle chart.

After BIs were placed, cycle development started with the suggested cycles of 65% RH for 30 minutes of condition time followed by charging to 5 mg/L (1800 ppm) and holding for 30 minutes of exposure. After testing a few other cycle times, the cycle that was finally used was 65% RH for 10 minutes of condition time and 5 mg/L for 50 minutes for a total cycle time of fewer than 90 minutes. Even though both isolators were different configurations (layout), the same cycle proved to be optimum for both isolators.

Once the cycle had been developed, it needed to be verified with a minimum of three consecutively successful runs demonstrating a complete kill of all BIs. An important note here is that this does not demonstrate an SAL for the isolator system. Since BIs with more than a million (10^6) bacterial spores were used, our answer to the sterility assurance question is, "Has demonstrated a complete kill of 10^6 Biological Indicators" The cycles were tested using 10^6 *bacillus atrophaeus* (ATCC 9372) Biological Indicator (BI) spore strips inoculated on paper and wrapped in tyvek. BIs were placed in 25 (packaging main chamber) 13 (air lock) and 21 (mix/fill) locations around each isolator, see Figures 4 and 5 for locations. Figure 6 shows a chart of the decontamination cycle in the mix fill isolator. It documents the RH monitoring and control and the concentration monitoring and control in real time. It also shows a cycle under the two hour requirement. The actual cycle time is 85 minutes.

6. Process Qualification, Unique to the Process Preformed in the Isolators

The most problematic portion of this validation was to maintain the appropriate non-viable particle counts. The mix/fill process involves mixing a dry powder and a gelled liquid in an ISO Class 4 isolator with less than or equal to 354, 0.5 and 0, 5 micron particles/M^3. This was largely accomplished by good aseptic technique, sample averaging and closely defining critical processing steps. Critical processing steps for mix/fill have been defined as the time that tissue is exposed to the isolator chamber.

7. Validation, Media Fill/Aseptic Fill, Again Unique to Process Preformed in Isolators

The media fill validation is simply running the process with a microbial growth media in place of our bone powder and HY then incubating the resulting packaged simulated product. Because the DBX putty is not a liquid, a custom media needed to be developed using both a sterile powder (TSB and CMC) and a liquid, (Water for Injection (WFI)) to create a reasonably translucent and viscous gel. Once developed, the media was validated to demonstrate growth promotion. All test samples demonstrated no growth.

8. Risk Assessment

The risk assessment was conducted using an ISO 14971 style assessment of the risks to patient/tissue. Using a team of experts familiar with the DBX putty process conducted in Biological Safety Cabinets (BSC) and a consultant familiar with aseptic processing in isolators, a Failure Modes and Effects Analysis (FMEA) work sheet was used to identify the potential failure modes, potential effects of failure, severity, potential causes of failure, occurrence, current controls and detection. Severity, occurrence and detection were each rated on a scale of 1 to 5 for each potential effect listed by the team. The product of the severity, occurrence and detection ratings is called a Risk Priority Number (RPN) which is used to rate the overall risk associated with each potential effect of failure. Generally higher RPNs require more/better controls.

All tissue, HY and packaging materials are packaged in sealed pouches which have been validated to prevent sterilant intrusion. Additionally, after the decontamination cycle the isolator chamber is aerated to less than the current eight hour permissible exposure limit (0.1 ppm), before any processing materials are exposed to the isolator chamber. In addition to the above, a CD residuals study was conducted on DBX putty which had been fully processed in the isolators. This study determined that there was no detectable level of CD in the DBX putty. This level of control results in an easily acceptable level of risk to both product and patent.

Discussion – Lessons Learned

Some lessons learned from the process include checking

materials used inside the isolators and the material of the isolator itself. Chlorine dioxide gas is an oxidizer. Its oxidation potential is 0.95.[24] Some materials chosen for equipment inside were incompatible with the number of cycles performed daily (two to three cycles per day). A few of the unpainted mild steel components in the heat sealer suffered some oxidation leading to progressively higher nonviable particle counts. This corrosion was initially fairly subtle, and once we had run enough cycles to cause more visible corrosion, it became apparent that not only the materials, but the surface finish of the materials were critical to achieving low nonviable particle counts; for example, the cold rolled steel shafts in the sealer have remained unaffected by exposure to chlorine dioxide except where slots and flats have been milled in them. Basically uncoated ferrous metals required paint or another coating; however, the aluminum extrusions used for the sealer have remained unaffected by chlorine dioxide exposure. Once we discovered what was going on, the affected parts were changed to more compatible materials (stainless steel and some plastics), or coated, the air exchanges were increased and the particle counts were reduced to acceptable levels.

Some lessons learned from the process include checking materials used inside the isolators and the material of the isolator itself.”

Another issue we discovered was that not only the volume of air going through the isolator is critical, but the direction as well. Our mix fill isolator brings air in from the top and extracts air through the floor and out the back of the bottom. This looked like a good way to extract particles as quickly as possible during the mixing operation. Unfortunately, this also requires that the floor of the isolator be kept clear to allow the air to flow through it. Since all the materials and equipment are locked in the isolator from start to finish, material location and work flow not only need to focus on efficiency of the process, but air flow through the isolator. We were able to overcome these limitations with improved work flow, strict attention to aseptic technique, and a trash basket built into the floor of the isolator.

Conclusion

MTF decided it needed to raise the standard of production to ensure product safety by processing and packaging its DBX putty inside isolators. Because of this a company wide effort to investigate isolators, choose the manufacturer, select a decontamination method, and validate the isolators and decontamination agent, and finally manufacturer product through the new process. It decided upon isolators used in conjunction with chlorine dioxide gas decontamination. The isolators eliminated the need for using 2.5 ISO 4 cleanrooms and provided true aseptic processing. The chlorine dioxide gas generator and isolators worked together to provide a simple and seamless systems integration. Both products met the needs for ease of use, design, flexibility, and decontamination cycle effectiveness and time.

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About the Authors

Nick Barbu is a Process Engineering Manager, leading a group of 10 engineers, designers and engineering technicians. The group is responsible for existing processes, process improvements, scale-up, and implementation of new product lines. He has managed various projects, largest being the isolators project (approximately $1.5 million). Barbu has 13 years of combined experience in medical device, pharmaceutical, and tissue banking. He holds a BS in mechanical engineering.

Musculoskeletal Transplant Foundation, Edison, New Jersey, USA.

Robert Zwick has more than nine years of medical device product and process validation experience with previous experiences as quality manager, technical manager, chemist and process engineer. He holds a BS in chemistry from Easter Michigan University. Since joining the Musculoskeletal Transplant Foundation in 2007, Zwick has validated medical devices, Human Cell and Tissue Products (HCTPs), isolator systems and automated disinfection and demineralization systems.

Musculoskeletal Transplant Foundation, Edison, New Jersey, USA.