Sterility testing of manufactured products is paramount in the fight against medicine born bacterial infection in patients.

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It is widely known that sterile production needs to be performed under HEPA filtration creating a grade A environment. For example, a Restricted Access Barrier system (RABs) helps assure that batch production is as particulate-free as possible and therefore also reduces the opportunity for viable particulate.

A sample batch is removed from the production run for sterility testing. Methods to do this have often been under the same conditions (i.e., laminar flow cabinet). However, more and more testing laboratories are opting to carry out sterility testing in bio decontaminated environments, often using an Isolator decontaminated with hydrogen peroxide.

The move from sterility testing within LAF safety cabinets to those in aseptic isolators has resulted in a marked decrease in false positive results as the following testimony from a National Biologics Institute states:
“We used to perform product sterility testing using a laminar flow hood in a Grade B environment. Most of our products are sterile filled in a Grade A area, state-of-the-art filling suite. We could not understand why we were obtaining several false positives in testing when using the flow hood. It took us months to investigate the root cause due to the difficulty in invalidating a sterility test. We re-looked at the sterility testing environment and decided to purchase a sterility test isolator from Extract Technology in 2014. To date, we have not had a false positive for sterility testing.

We will not go back to performing sterility testing in a Laminar flow hood.

The H₂O₂ sterilization reduces by log 6 Bacillus spores and the isolator creates an ISO 5 (4.8) Grade environment which eliminates any contamination entering the testing environment. After the use of the sterility test isolator, we have not had one false positive. We are testing approximately 2000 batches a year. From experience, using an isolator will reduce the number of false positives in sterility testing.”

Hydrogen peroxide (H₂O₂) has been used as a kill bacteria for 20+ years and is recognized by the FDA and other regulatory agencies as a means of bio-decontamination.

Antiseptics and Disinfectants: Activity, Action, and Resistance
Gerald McDonnell and A. Denver Russell
Peroxygens - Hydrogen peroxide

H₂O₂ is a widely used biocide for disinfection, sterilization, and antisepsis. H₂O₂ is considered environmentally friendly because it can rapidly degrade into the innocuous products: water and oxygen.

H₂O₂ demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts and bacterial spores. Higher concentrations of H₂O₂ (10 to 30%) and longer contact times are required for sporicidal activity, although this activity is significantly increased in the gaseous phase.

While the price of this testing environment may seem cost-prohibitive, the proven results offset the cost and time one would spend on lost batches, justifying a sound investment.

Customers Want Repeatable, Reliable and Faster Decontamination

The Uni-Directional AirFlow (UDAF) SteriPharm is an EU GMP Grade A (ISO14644-7:2004) aseptic environment in which various sterile tasks can be carried out. In this instance, the system was being used for sterility testing. While a unidirectional airflow is paramount for sterile production, a single pass turbulent flow system is also accepted (US Pharmacopeia 1208) for sterility testing.

To begin, the main chamber is loaded with items to be tested. In this case, media tested is Merck Steritest™ packs for use with the advanced Symbio Flex Steritest unit.

Cycles are developed around specific loads, therefore it’s important to load the chamber in a repeatable way. For example, racking/shelving is designed specifically to hold a certain product amount and type, whether samples or test media. It is recommended each load configuration is photographed so any operator can easily repeat.

Additional media containers equate to additional surface area. Additional containers of an absorbent
material, such as plastic, may all have a negative effect on the decontamination performance of the pre-programmed cycle.

Further cycles can be programmed following load development using a BI challenge. It should not be assumed the same program will work for different load variants.

Once the samples, media bottles and implements are loaded, the visor can be closed and the cycle started.

**View of load ready for gas cycle development**

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Faster

Complete decontamination cycle times are a common discussion point when selecting a suitable system. Many factors determine this, including size of the isolator chamber, material of construction, injection rate, and duration, not to mention the variety of the load. Aeration is the longest stage of the bio-decontamination cycle which could happen over a period of hours. Designing a system that most efficiently removes the hydrogen peroxide is key to improving this time.

While running a “lights-out” cycle in preparation for next-day testing satisfies some clients, others who have a high production throughput require quicker cycle times.

Load specific Gas Cycle Development (GCD) must be completed to ensure, primarily, that a $10^{-6}$ kill is not only achieved, but is repeatable and that the cycle times can be reduced as much as possible within a safety margin. Each machine must be challenged with BIs to ensure this is achieved.

The four stages of the gas cycle are as follows, each scrutinized to reduce times for a major pharmaceutical manufacturer in Russia.

**Conditioning**

Vapor Phase Hydrogen Peroxide (VPHP) works more efficiently in a dryer environment. A fully integrated dehumidification is included, allowing for better suspension of the VPHP and reduced dilution of the 35% concentration media used. In parallel with this phase, other operational systems are primed, such as the gassing generator and trace heating in the delivery pipework.

**Bio-Decontamination (Injection)**

Hydrogen peroxide, in vapor form, is injected via nozzles into the chamber to effectively disperse the VPHP throughout the chamber.

A two-stage process, the chambers are first injected with VPHP over a period of minutes. Then, the recirculation fans distribute the gas through the entire system.

**Dwell (Kill)**

The dwell or kill phase is then entered, allowing the H$_2$O$_2$ to achieve a 6log reduction in the bacterial spore population.

**Aeration**

The longest of the four cycle stages, aeration efforts focus on ultimately reducing the overall gas cycle time from around five hours to a targeted three hours.

Off-gassing plays a large part in extending this stage. Certain materials, such as plastics, absorb the VPHP during dwell and release them slowly during aeration. The viewing window yielded one of the largest savings. This small front-end investment provides a measurable savings throughout the life of the system.

It was also found that in aeration, the VPHP was condensing in the piping system. This meant the condensate droplets needed to evaporate, thus giving an elevated ppm reading throughout the aeration stage. Again, minor adjustments made a large
difference in the overall aeration time.

Graph highlighting each gassing cycle phase

With these changes, Extract Technology achieved entire system decontamination in less than 90 minutes. This was executed with a 1.755m³ [62 cubic-foot] interior volume isolator made up of a 4-glove working chamber and a 1m³ [35 cubic-foot] transfer chamber which can be gassed separately if required in 30 minutes (with load).

Based on a customer-designated load, half of the original target was achieved and completed within the required margin of safety.

How do you take custom quality and intelligence and make it standard?

As medicinal production ramps up in the pharmerging markets—for domestic use and global export—both tried and trusted European and US manufactured products are in demand. Companies with years of experience in delivering cGMP-compliant equipment are favored, as successful FDA audits are vital in competing for product export.

How can years of experience be applied to a standard system, all while maintaining the necessary quality to ensure patient, operator and environment safety within a viable solution?

Several challenges should be considered beyond price point, equipment and service quality.

The heart of standardization and operational efficiency is a system built and tested for successful repetition on the same equipment, ultimately reducing design cost and time.

Incorporating a series of options that cover most process applications can aid in adapting a standard system to a specific process requirement. Such options could include:

- Rapid Transfer Ports (RTPs)
- Electrically actuated raise and lower systems for performing testing in either standing or seated position
- Viable and non-viable particle counters
- Chart recorder for full 21CFR Part 11 compliance
- Full cycle development in a dedicated test facility

All variables considered, it is imperative to balance cost savings, without compromising the quality and reliability that is vital in the production and testing of sterile products.

For more information:

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