

## H2O2 Sterilization for Freeze Dryers

The need for sterilization of freeze dryers has grown over the past decade. Many methods of sanitization and sterilization have been used in freeze drying, each with its own advantages and disadvantages, they include:

Alcohol  
Formaldehyde  
eTO  
Radiation  
UV  
Steam sterilization  
H2O2

The most common means of sterilization today is steam sterilization. The process of steam sterilization raises the temperature and pressure of the freeze dryer to 121C and 25PSI for a period of time and number of cycles. Although the industry is comfortable with its use steam sterilization adds significant cost and complexity to a freeze dryer installation and takes significant cycle time.

Freeze dryers typically operate at very low temperatures and very low vacuums and do not require a positive pressure rating. To accommodate steam sterilization the vessels must be pressure and temperature rated which adds significant cost, often as much as doubling the cost of the equipment. A steam generator and stainless steel piping are also required.

Due to the significant cost associated with steam sterilization and since existing freeze dryers cannot be converted to steam sterilizable, an alternate method such as H2O2 sterilization should be considered.

### How does H2O2 sterilization work?

HPV technology has existed for a decade and utilizes the microbicidal power of free radicals released by hydrogen peroxide (H2O2). Over 85% of the aseptic process isolators in the world use HPV technology as their decontamination agent of choice (ISPE, 2005). In addition to freeze dryer sterilization, HPV can be introduced into a variety of systems such as isolators, rooms, restricted -access barrier system (RABS), filling lines, and process/ manufacturing areas using different configurations.

The technology works by producing HPV through flash evaporation of an aqueous solution of H2O2. The process is effective at ambient temperature and relative humidity so no special preconditions such as de/pre-humidification is necessary.

HPV is introduced to the enclosure evenly, and that each surface is comparably exposed to the HPV. The equipment lays down approximately 1 micron of H2O2 onto exposed surfaces where microorganisms may reside. The microorganisms themselves act as nuclei for the formation of microcondensation, thus speeding action of the process.

The process is controlled from outside the enclosure via a control computer with a PLC and provides real time feedback of cycle progress. The room or equipment being exposed to HPV is sealed and monitored by electrochemical hand-held HPV sensors to ensure that there is no leakage and that the level of HPV has returned to safe environmental levels after the cycle.

### Quick Definitions

**Sanitize** – the cleaning of pathogenic microorganisms from public eating utensils and preparation surfaces

**Disinfect** – to reduce the number of viable microorganisms present in a sample

**Sterilize** - the killing of 'all' microorganisms in a material or on a surface. Generally accepted as a 6 log reduction biologic activity

The object of sterilization is a 6 log reduction in colony counts and is tested using biological indicators (BI's). Biological indicators are spore strips that require good penetration for a kill. An example is *Bacillus Stearothermophilus* (spores).

Post decontamination the HPV is catalytically converted into water vapor and oxygen. The HPV can either be completely neutralized in the specified area using powerful aeration units or in conjunction with the building's heating ventilation and air conditioning (HVAC) system.

**H<sub>2</sub>O<sub>2</sub> Compatibility** Hydrogen peroxide has been utilized for a decade or more in a wide variety of facilities and has proven compatible with materials including sensitive electronics, microscopes, stainless steel, and HEPA filters.

### **Typical H<sub>2</sub>O<sub>2</sub> process**

The hydrogen peroxide vapor process happens according to the four operating stages detailed below:

- 1. Conditioning:** Approximately five to ten minutes are allotted to allow the hydrogen peroxide vaporizers within the gas generator(s) to thermodynamically stabilize at temperature of approximately 120°-130° C.
- 2. Gassing:** Readily available medical grade liquid hydrogen peroxide, nominally 30–35% w/w, is flash evaporated within the gas generator(s) and dynamically distributed throughout the target area. This is enhanced with nozzle(s) that simultaneously rotate vertically and horizontally in order to evenly distribute the vapor throughout the area at velocities approaching 100 ft/sec.
- 3. Dwell:** Hydrogen peroxide vapor is allowed to “dwell” inside the target area for the designated time frame to ensure full bio-deactivation. This element of the process allows the target area(s) to receive additional exposure to the hydrogen peroxide vapor ensuring that a successful result is achieved.
- 4. Aeration:** Vapor is removed from the target area(s) by making use of the facility's HVAC system, and /or aeration units which rapidly catalytically convert the vapor into oxygen and water. The advantage of a separate aeration unit is that it provides a means of removing the vapor in the event the facility's HVAC system does not have suitable dampers or access to their controls.

### **H<sub>2</sub>O<sub>2</sub> Equipment**

The latest H<sub>2</sub>O<sub>2</sub> generators eliminate the need for pre/de humidification, water ring pumps, and special sensors inside the equipment. A catalytic system is used to neutralize the H<sub>2</sub>O<sub>2</sub> after the sterilization process is complete, thus eliminating the need to monitor any exhaust. In addition, the newest systems are meant to be portable and flexible enough to decon equipment plus entire rooms or suites.

### **Process Development and Validation**

The H<sub>2</sub>O<sub>2</sub> vapor generator/Freeze dryer manufacturer can help develop a process and validate the system which includes a Factory Acceptance Test (FAT), Installation/Operational Qualification (IQ/OQ), Gas Cycle Development (GCD), and Performance Qualification (PQ). This series of events includes the following to properly validate the system:

Documentation checks, Post Delivery Inspection, Supplies Checks, OQ Certificate Checks, OQ Function Tests, Pre-gassing surface temperature checks, Gas distribution study with Chemical Indicators (CI's), Biological Indicators (BI's) location mapping, and Refining of Gassing, Conditioning, and Aeration time. .

### **Retrofit to existing equipment**

H<sub>2</sub>O<sub>2</sub> can often be retrofit to an existing freeze dryer and requires minor modifications to new systems. A valved port for the H<sub>2</sub>O<sub>2</sub> source and one for the exhaust are the minimum requirements. These connections need to be of sufficient size to allow proper flow, such as a 1” ID tube. Typically the H<sub>2</sub>O<sub>2</sub> enters the freeze

dryer at the highest point and is removed at the lowest point. More advanced H<sub>2</sub>O<sub>2</sub> sterilization systems provide all the necessary features built-in to control the sterilization process.

### **Conclusion**

There are numerous ways to decontaminate but HPV technology seems to be the way of the future. It has proven biological efficacy, excellent material compatibility, and numerous applications throughout the pharmaceutical/bio-tech industries. In the present and future, these technologies will continue to help produce safer and more efficient processes.

### **Further Information**

If you are interested in further information or have additional questions, please contact the authors:

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## Summary Table

Method	Advantage	Disadvantage	Comment
H2O2	<ul style="list-style-type: none"> <li>• Easy to retrofit</li> <li>• Low cost of equipment</li> <li>• Lower cost of operation</li> <li>• Short cycles (&lt;3 hrs)</li> <li>• Validatable</li> <li>• 'Safe'</li> <li>• No residue</li> <li>• Proven – FDA approvable</li> <li>• Effective on endospores</li> <li>• One generator can be used on multiple systems</li> </ul>	<ul style="list-style-type: none"> <li>• Not as widely used as steam since</li> </ul>	<ul style="list-style-type: none"> <li>• 30% with water for medical grade</li> <li>• Multiple sources</li> <li>• By-products are water and oxygen</li> </ul>
Steam	<ul style="list-style-type: none"> <li>• Widely accepted by FDA</li> <li>• Validatable</li> </ul>	<ul style="list-style-type: none"> <li>• Expensive to implement</li> <li>• Requires pressure rated vessel</li> <li>• Requires steam generator</li> <li>• Safety</li> <li>• Long cycles (6-9 hours)</li> <li>• Can stress chamber and welds</li> </ul>	<ul style="list-style-type: none"> <li>• Wet steam is used</li> <li>• &gt;121C for a period of time and number of cycles</li> </ul>
IPA 70%	<ul style="list-style-type: none"> <li>• Inexpensive</li> <li>• Simple</li> <li>• No residue</li> </ul>	<ul style="list-style-type: none"> <li>• Not penetrating</li> <li>• Not sterilization – does not kill endospores</li> <li>• Not consistent and difficult to validate</li> </ul>	<ul style="list-style-type: none"> <li>• Good for applications where sterility is not required</li> </ul>
Formaldehyde	<ul style="list-style-type: none"> <li>• Long history of use</li> <li>• Inexpensive</li> </ul>	<ul style="list-style-type: none"> <li>• Leaves residue</li> <li>• Control of concentration difficult</li> <li>• Hazardous – suspected carcinogen</li> </ul>	<ul style="list-style-type: none"> <li>• Formalin is a 35% solution of formaldehyde</li> </ul>
EtO	<ul style="list-style-type: none"> <li>• Inexpensive</li> <li>• Demonstrated effectiveness</li> </ul>	<ul style="list-style-type: none"> <li>• Leaves residue</li> <li>• Difficult to control</li> <li>• Hazardous – suspected carcinogen</li> <li>• Venting/Neutralization</li> </ul>	

## References:

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