Development of Vaporized Hydrogen Peroxide (VHP) Decontamination Cycle for a Syringe Isolator

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Abstract *— A parenteral syring filling line inside a closed cabin isolator was installed in an existing manufacturing space. To increase sterility assurance the isolator must be effectively biodecontamined by means of Vapor Hydrogen Peroxide (VHP) to provide a minimum of 6 log-reductions and a residual concentration of VHP of less than 1ppm. During the cycle development phase the operating characteristic and parameters for the decontamination were tested and optimized following the proposed methodology in this article. Final adjustment of the injection quantities was based upon results of the worst case biological indicator tests performed during the study. Based on the results, parameters and validation testing were established to provide for complete destruction of all biological indicators.*

Key Terms *— H_2O_2 decontamination, cycle development, biological indicator (BI), chemical indicator (CI)*

INTRODUCTION

A new parenteral syring filling line inside a closed cabin isolator was installed in an existing manufacturing space. The intention to enclose this filling line by means of a barrier isolator is to reduce product contamination possibilities during the filling process due to the surrounding environment and personnel. In order to maintain product protection as needed, this isolator must be effectively cleaned and decontaminated.

OBJECTIVE

The challenge is to develop an adequate decontamination cycle and demonstrate that this decontamination cycle is capable to provide a minimum of 6 log-reduction of a suitable biological indicator organism (*Geobacilus stearothermophilus) in the isolator cabin and demonstrate a residual concentration of hydrogen peroxide of less than 1.0 ppm at the completion of the cycle.*

Isolator Description

The barrier Isolator is a standalone system that has the following components sections:

- The main cabin that contains the filling/stoppering machine, infeed and discharge airlocks, return air ducting via the double wall returns, glove ports and rapid transfer ports. The infeed and discharge air lock that is fitted to either end of the barrier isolator cabin corresponding to the infeed and discharge conveyor sections of the syringe filling and closing machine. This is the area where the product is going to be exposed during the filling process.
- The laminar flow section, which is the section provided for mounting to the top of the isolator cabin. The purpose for mounting to the top of the isolator laminar flow section is to provide unidirectional airflow to the working areas of the barrier isolator cabin. This designation includes all necessary filters, fans dampers and ductwork.
- The heating, ventilating, and air conditioning (HVACs) section, which consist of an air handling unit and a dehumidification unit. The air handling unit (AHU) is provided with the system intended to supply air to the laminar flow section for over pressurization and temperature control. The AHU is mounted remotely. This designation includes all necessary fans, filters, coils, and dampers whether they are mounted with the package

equipment boundary or remotely. The dehumidification unit is provided with the system for purposes of dehumidifying the barrier isolator cabin prior to and during the decontamination process.

- The bio-decontamination section which provides vaporized hydrogen peroxide (VHP) to the main cabin and piping of the barrier isolator. This system includes all equipment necessary for the controlled transfer of the vapors of the liquid H_2O_2 and throughout the system as required. This Isolator unit is equipped with a hydrogen peroxide dispensing unit (Safe VAP). This Safe VAP will convert the hydrogen peroxide dispensing unit (Safe VAP). This Save VAP will convert the hydrogen peroxide (VHP) that will be distributed uniformly through the Isolator chamber in order to reduce the microorganism concentration.

METHODOLOGY

Qualification and validation of the bio-decontamination process follows a two phase approach: Cycle Development (CD) and Cycle Validation (CV). With the aid of practical experimental data this paper presents in detail the individual stages involved in the method proposed for decontamination cycle development, and interpretation of the results and their implications for the process parameters.

During cycle development phase the objective is to determine the materials to be used as sporicidal agent, loading patterns, sampling locations and exposure time. Testing performance during cycle development includes air flow verification testing, temperature, humidity, residual chemical concentration in the system and worst case positions using biological indicators (BIs). AS the final step of cycle development, a cycle verification test is performed to demonstrate cycle efficacy using proposed routine production parameters, and to determined cycle validation will be performed.

During the cycle validation phase the oerating characteristic and parameters for the decontamination are tested, to confirm the final cycle parameters to be used. During this phase triplicate testing of the proposed routine cycle will be performed to demonstrate reproducibility. Successful completion of this phase validates the cycle for routine production use.

In VHP surface decontamination, the overall bacterial reduction is obtained from the release of gaseous hydrogen peroxide (H_2O_2) and the effect of the lethal dose over time. There were three concentrations of hydrogen peroxide available for the study (31%, 33% and 35%). Due to time and material constrains it was decided to use the 35% concentration to ensure successful results, although the manufacturer recommended a 33% concentration. Vapor hydrogen peroxide release was subdivided into four cycle phases already programmed in the equipment as follows: [2]

Phase 1 Drying

- Leak Test – a leak test of the unit is automatically performed to verify integrity of the cabin and piping prior to the start of the cycle. All cabin door and the gas tight flaps are closed; filtered compressed air is used to increase the cabin pressure to fall to the set value is measured and recorded. The intention of this phase is to ensure proper isolation of the chamber from the surroundings in order to avoid any hydrogen peroxide leak to the environment.
- Drying – during the drying phase the system is dried and heated to achieve consistent temperature and relative humidity conditions at the start of the conditioning phase to improve the absorption of the hydrogen peroxide. Isolator air recirculated through the desiccant wheel dryer to reduce the relative humidity percent (%RH) to less than the set point value. During this phase a reduce quantity of air is drawn from the surrounding clean room environment for regeneration of the desiccant wheel and exhausted to the outside.

- Heating of the evaporators and distribution piping is performed in preparation for the conditioning and bio-decontamination phases of the cycle. This heated air from the vaporization section also increases the cabin temperature to the minimum set point value.

Phase 2 Conditioning

- During conditioning phase H_2O_2 injected at a higher rate to obtain the required levels within the isolator. Liquid peroxide (Solvay Chemicals, 35% H_2O_2 solution) is vaporized in the Safe VAP unit via filtered compress air venture injection nozzles and vaporizing elements. The vaporized peroxide is fed into a dry air stream provided by a separate de-humidification unit, and then delivered via the distribution piping to the isolator ductwork. Multiple injection point area is provided to ensure uniform H_2O_2 concentration throughout the isolator cabin and airlocks. During conditioning a reduce quantity of air (relative to the production mode) is drawn from the surrounding clean room environment and exhausted outside via the remote HVAC unit. Overpressure of the system relative to the surrounding clean room is actively controlled at the set point value.
- Dispensing of H_2O_2 in the system is gravimetrically controlled. A weighing scale is located in the H_2O_2 cabinet to measure and record the rate of dispensing (g/min) and total weight dispensed. The system is equipped with an alarm in case the quantity of H_2O_2 at the start of the cycle is insufficient to complete the cycle.
- The presence of H_2O_2 inside the cabin is detected during conditioning and bio-decontamination by means of a sensor. An alarm occurs if the minimum level is not reached.

Phase 3 Bio-decontamination

- During the subsequent decontamination phase, the H_2O_2 injection is maintained at a constant flow lower rate, to maintain cycle lethality. During bio-decontamination a reduced quantity of air (relative to the production mode) is drawn from the surrounding clean room environment and exhausted outside via the remote HVAC unit. Overpressure of the system relative to the surrounding clean room is actively controlled at the set point value.
- The rate of injection and total quantity injected are monitored and recorded for both the conditioning and bio-decontamination phase of the cycle.

Phase 4 Aeration

- During the aeration, an increase quantity of air is drawn from the surrounding clean room and exhausted to the outside, in order to reduce H_2O_2 levels inside the system (filters, surfaces, injection and piping) to the specified concentration (<1ppm). Air inside the cabin is sampled at regular time intervals using a sensor installed in the cabin to ensure attaining the specified concentration. The equipment has the capacity to measure and report hydrogen peroxide levels. Overpressure of the system relative to the surrounding clean room in again actively controlled at the set point value. Figure 1 illustrates the decontamination effect as a function of a cycle phase.

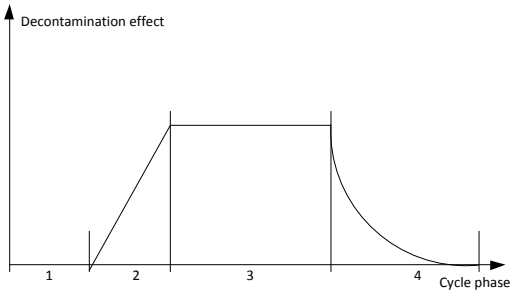

Figure 1
Decontamination Effect as a Function of a Cycle Phase

As previously mentioned, these four cycle phases were already programmed phases has control parameters to monitor equipment functions already established. The key parameters to monitor each of the cycle steps are listed in Table 1.

Table 1
Key Parameters to Monitor the Cycle Steps

Cycle Phase	Parameters [Units]	Effects
Drying	Air Humidity [%RH] Air Temperature [ºC] Air Velocity [m/s]	Reproductibility of the decontamination cycle preparing the surfaces for better absorption of the killer agent (Hydrogen peroxide)
Conditioning	Initial Quantity of Aqeous H_2O_2 per volumen (ql), [g/m3] Injection Rate [g/min] Time [min]	Initial exposure to the killer agent (start dispensing the solution)
Decontamination	Quantity of H_2O_2 [g] Infection Rate [g/min] Time [min]	Ensure adecquate solution coverage to maintain stability of the bacterial reduction rate proposed.
Aeration	Time [min]	Elimination of residues of the killer agent used in order to minimize chemical contamination of the product and to increase safety levels for the operators using the equipment.

Cycle Development

As previously discussed, the purpose of the isolator cabin is to enclose the filling equipment (filler and stoppering system) from its surroundings in order to minimize product exposure to the environment and reduce dramatically contamination risk possibilities. In order to grant isolation from the surrounding environment the isolator cabin is equipped with its own Air Handling Units and associated High Efficiency Particulate Air Filters.

For this particular study the selected sporicidal agent was vaporized hydrogen peroxide. This sporicidal agent was recommended by the cabin isolator manufacturer considering the design of the Safe VAP unit included. There were two sporicidal agents that were compared prior to make the decision: formaldehyde and hydrogen peroxide. Considering the design of the Safe VAP unit and the AHU that serves the isolator cabin they recommended the hydrogen peroxide since it requires no fan pulses to be eliminated from the chamber, the formaldehyde fumigation cycles required a high humidity levels while the hydrogen peroxide proved a high material compatibility with a wider range of normally use materials.

The interaction of vaporized hydrogen peroxide concentrations and temperature had been widely investigated. The rate of spore inactivation achieved in these studies is proportional to gas concentration. These studies demonstrated that for a given concentration of vaporized hydrogen peroxide the rate of spore inactivation was slower on warmer surfaces that at cooler surfaces [2]. These observations support the concept to adjust the sterilant exposure time base on isolator temperature distribution studies and the percent saturation of hydrogen peroxide gas concentration. Considering the design of the isolator and the construction materials of the equipments enclosed within the isolator chamber the determination of the "worst case" positions must be performed. These positions are the ones to be sampled during validation of the biodecontamination cycle ensure adequacy of the cycle. The efficacy of the selected sporicidal agent must be challenged assuring microorganisms reduction in these positions.

The purpose of the biodecontamination process is to reduce an existing microbiological contamination load by a define level. This requires an understanding of the applied process and the inactivation performance. To establish the correlation between the process parameter and microbiological inactivation, the following steps were fallowed during cycle development:

1. **Identification of possible worst case positions by checking air flow** – During this stage the recommendation is to use generated aerosols to visualize airflow patterns within the isolator system in order determine "worst case" positions (gaps where air flow does not directly contact surfaces inside the isolator cabin). A smoke generator using de-ionized water can be used to produce localized smoke for visualization of airflow patterns during testing. This study is documented using a video camera.

2. **Identification of possible worst case positions by temperature mapping** - Using by calibrated instruments the temperature distribution and surface temperatures inside the isolator will be mapped. This will be performed to determine temperature variations and impact regarding absorption and evaporation rate of the selected sporicidal agent considering the different construction materials present in the filling line (stainless steel, glass, Teflon, tyvex, plastic). A maximum and minimum value of temperature must be determined as well as the temperature variations on surfaces within the isolator cabin.
3. **Identification of possible worst case positions with chemical indicators (CI's)** – Chemical indicators, plastic laminated paper strips coated with indicator ink that demonstrates a gradual color change from yellow to violet-gray upon exposure to H_2O_2 gas, which was the selected sporicidal agent, should be located through the isolator in order to determine time necessary for the sporicidal agent to penetrate that specific location. This information will serve to assess hard to reach locations "worst case" positions and minimum time that will be used as baseline to calculate the exposure time to ensure a successful cycle. Since the strips are going to change color once exposed to the sporicidal agent it is recommended to place them in easily visible locations from outside the isolator and have the adequate amount of persons surrounding the isolator in order to record the time lapse required for each strip to show the color change.
4. **Identification of possible worst case positions with biological indicators (BI's)** – Spore-inoculated carriers (Biological Indicators) should be placed in the same locations were the chemical indicators were placed to biologically monitor the efficiency of gas distribution and to evaluate cycle lethality. *Geobacillus stearothermophilus* is commonly used as a challenge organism for sterilization validation studies and periodic check of sterilization cycles [5]. The biological indicator contains spores of the organism enclosed on tyvek paper strips. The tyvek paper serves as an additional challenge for the sporicidal agent penetration. These paper strips once exposed to the sporicidal agent are incubated. A color and/or turbidity change indicates a non satisfactory results for the sterilization process, (this color change is associated with the growth of the spores); no change indicates sterilization conditions were achieved. For this test it is recommended to intentionally reduce the cycle lethality so as to produce positive BI's at several locations within the working section of the isolator. Positions for which the biological indicator results are positive are proven to be worst case related to cycle lethality. At this stage the cycle parameters can be gradually modified until all BI's are negative for microbial growth.
5. **Determination of D-Value** - Whenever Biological Indicators (BI's) are used to demonstrate sterilization it is recommended to determine the D-value of the biological indicators used in order to confirm the suitability of the BI's used for the qualification. The D-value refers to decimal reduction time of pathogens microorganisms when exposed to sterilization processes (such as exposure to heat or bio-decontamination) [4]. The time required at a certain temperature or time to kill 90% of the organisms being studied. Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. The population number has been reduced by one decimal place in the counting scheme. Generally, each lot of a sterilization-resistant organism is given a unique D-value. When referring to D values it is proper to give the temperature as a subscript to the D. For example, hypothetical organism is reduced by 90% after exposure to temperatures of 300 degrees Fahrenheit for 2 minutes. Thus, the D-value would be written as D300F = 2 minutes.

D-value determination is often carried out to measure a desinfectant's efficiency to reduce the number of microbes, present in a given environment. In this particular case since time will be the parameter instead of temperature it is recommended to determine the D-value using a method derived from the Limited Spearman-Karber Method (LSKM, USP29-NF24). For this test it is recommended to place a large number of BI's in a tightly sealed container inside the isolator, in a central location that would permit easy handling for exposing and retrieval of the BI's. A bio-decontamination cycle will be started and at the beginning cycle will be started and at the beginning of the decontamination phase, all the BI's are going to be removed from the gas tight container and exposed in the main cabin. A specific amount of BI's are going to be removed at regular recorded time intervals during the exposure phase, transferred to nutrient growth media and then incubated. BI's are going to be monitored for growth and the data will be recorded.

6. **Cycle verification with BI's** – Once the previously described tests are completed, the cycle efficacy and suitability of it must be determined. Based on the results of the previous test section, a final BI placement map should be determined. A single cycle verification run will be performed with one BI located at each determined test position. Upon completion of the cycle, BI's will be removed from the isolator main cabin, transferred to growth media and incubated at 57.5º ± 2.5ºC during seven days to be then observed for growth.
7. **Evaluation of the aeration time** – This test is recommended to determine the time required during the aeration phase to reduce the concentration of vaporized hydrogen peroxide (VHP) below the required safety level of <1ppm [3]. For this test it is recommended to use Draeger testing tubes for evaluation of the residual concentration of hydrogen peroxide with hand pump, for measurement of airborne VHP in the range of 0.1 to 3ppm. The test tubes must be placed in a suitable monitoring location immediately upstream (within the airflow) form a centrally located isolator return duct. Once the desired concentration levels are reached according to the Draeger test tubes results, the time will be recorded and set as the aeration time needed for routine production use.

Summary of Results

The results for the test described above are summarized in order to set the baseline parameters determined to continue to the next and last stage of the qualification of the bio-decontamination cycle which is the cycle validation as described in the Methodology section.

- Identification of possible worst case positions by checking air flow – The test that consisted in the use of generated aerosols to visualize airflow patterns within the isolator system in order to determine "worst case" positions (gaps where air flow does not directly contact surfaces inside the isolator cabin) by means of a smoke generator using de-ionized water was performed. One the constrain that was experienced was during the execution of the smoke study at dynamic conditions. When the test was executed it was experienced was the activation of alarms that automatically stop the isolator due to the sensor motion devices. Every time the operators got close to the isolator cabin the filling process was interrupted due to the alarm. After several trials it was decided that equipment re-programing was necessary to solve this issue. In order to alter the equipment program, it was necessary to contact the manufacturer hence this was a time consuming activity and a delay of the project was experienced.

  After completing the test it was noticed that the evaluation of the results was subject to interpretation, there were different opinions when the recorded results (video) were

watched by different personnel from different areas (multidisciplinary team). As a result of these different opinions, the positions identified as possible worst case were subjected to further testing to verify adequate cycle efficacy at these locations. A total of nineteen positions were identified as possible worst case. Refer to Table 2 for the cycle parameters used during the air flow verification of worst case positions. The inclusion of BI's was necessary to confirm cycle efficacy at these locations.

Table 2
Cycle parameters air flow worst case verification test

Parameter	Value
Air Veocity (m/s)	0.18 m/sec
Differencial Pressure (Pa)	17Pa
Temperature (ºC)	27.3 ºC
Humidity (%rH)	50.1%

- **Identification of possible worst case positions by temperature mapping** – For this test a total of twenty locations were monitored inside the isolator cabin. After this test the coldest surface as well as the hottest one was identified. A difference of 3.93ºC between the coldest 26.44 ºC and the hottest 30.37 ºC temperature was reported.
- **Identification of possible worst case positions with chemical indicators (CI's)** - For this particular test, ninety-one chemical indicators were spread through the isolator cabin in order to monitor color change. One constrain identified when performing the test was related to the chemical indicators placement positions. Some of these indicators were located in places that were hard to see from the watching point determined. The test was repeated placing chemical indicators in easily visible locations and the time was monitored. In order to determine the possible worst case positions, those showing color change in a period longer than fifteen minutes were considered. The test identified thirty-six possible worst case locations.
- **Identification of possible worst case positions with biological indicators (BI's)** – A minimum of eighty locations in the working sections of the barrier isolator were selected; three BI's were placed at each location. Locations designated as worst are positions based on airflow verification, temperature mapping and chemical indicators were included, along with representative "good" locations from each of these tests for comparison. Additional locations to bring the total to a minimum of eighty were selected upon equipment review. Three bio-decontamination cycles were necessary in order to complete the test. A range between 600 to 900 g of hydrogen peroxide in the conditioning phase has been determined to be the target range to obtain the desired results of 5 to 20 positive BI's. The run was executed at reduced parameters of 600 g. This resulted in growth from 150 out of 273 possible positions. After increasing the parameters to 900 g for the conditioning phase, there was only one positive growth out of the 273 BI's. Position number 3 located inside the stopper cover was positive. For the third run the parameters were scaled back to 800 g again, the results yielded only one positive BI. Position number 17 located on the stopper Rapid Transfer Port (RTP). The two positions for which the biological indicator results were positive are considered to be worst case related to cycle lethality. Table 3 summarized the results of the three cycle runs for the worst case biological indicators (WCBI).

Table 3
Biological Indicator Results

Test Run	Total No. of BI Posi-tions	No. of Posi-tions with 3 positive BI's	No. of Positions with positive BI's	No. of Positions with 1 positive BI's	No. of Positions with 0 positive BI's
WCBI 1	91	40	11	7	33
WCBI 2	91	0	0	1	90
WCBI 3	91	0	0	1	90

- **Determination of D-Value** – The D-value of the Biological Indicators (BI's) used for the studies was calculated to be 0.50 min (0.48 to 0.61 min).
- **Cycle verification with BI's** – Based on the results of the previous tests, a final BI placement map was determined. This map will be later on used for the Cycle Validation run. Cycle parameters were likewise established based on previous test results and was intended to provide an adequate margin of safety for routine production cycles. A single verification run was performed with one BI located at each test position. There was a hundred percent destruction of all Biological Indicators used in the isolator.
- **Evaluation of the aeration time** – This test was performed using Daeger testing tubes for evaluation of the residual concentration of the hydrogen peroxide with hand pump. The testing tubes with the hand pump were placed in a suitable monitoring location and samples were collected every 30 minutes from the start of the aeration phase. When the sample concentration showed results of less than 1 ppm, the sample interval was decreased to one sample every five minutes until three consecutive readings of <1ppm were obtained. The test determined that 2 hours were required to bring the hydrogen peroxide levels to <1ppm. Worst case locations for BI's were determined considering airflow visualization, temperature mapping, equipment geometry, chemical indicators, risk assessment of the equipment and worst case BI challenge performed with reduced cycle parameters. For each location determined a written rationale including the test used to identify that location was included as part of the study.

The cycle parameters set points for each of the test elements were adjusted as required during each test performed. Final adjustment of the injection quantities was based upon results of the worst case biological indicator tests performed during the study. Some tests were run using reduced parameters in order to obtain positive results at some locations to finally demonstrate the six log reduction process for all the locations tested.

Refer to Table 4 for different cycle parameters used for each test execution.

Upon completion of the Cycle Development Phase (CD), the Cycle Validation Phase was initiated. During this phase reproducibility of the proposed results was demonstrated. Three biodecontamination runs were performed using the cycle parameters established at the conclusion of the cycle development study. The biological indicators were placed in the locations determined as part of the cycle development.

The results observed for the three runs performed were satisfactory. All the BI's located within the isolator were killed during each of the three runs confirming the adequacy and reproducibility of the biodecontamination cycle selected.

Table 4
Parameters Used for the Different Test

Parameter	CI Run	WCBI 1	WCBI 2	WCBI 3	D-value	BI Verf.
Leak Test Cabin (s)	>72	>72	>72	>72	>72	>72
Leak Test Comple System (s)	>48	>48	>48	>48	>48	>48
Set value air temp Isolator (°C)	≥25	≥25	≥25	≥25	≥25	≥25
Air Velocity (m/s)	0.2±0.1%	0.2±0.1%	0.2±0.1%	0.2±0.1%	0.2±0.1%	0.2±0.1%
Set Value Air humidity % (RH)	20	20	20	20	20	20
Set injection rate C Phase (g/min)	40	40	40	40	40	40
Set H_2O_2 (C Phase) (g)	350	600	900	800	1000	1000
Set quantity H_2O_2 (Biodec. Phase) (g/min)	25	25	25	25	25	25
Set quantity H_2O_2 (C Phase) (g)	200	125	300	250	500	500
Total qty H_2O_2 (C/BD Phase) (g)	550	725	1200	1050	1500	1500
Aeration (min)	90	90	90	90	90	150

CONCLUSION

In order to develop an adequate decontamination cycle the knowledge of the equipment and how it works is essential. This knowledge served to determine the specific tests that were required to establish the worst case locations and adequate parameters for the biodecontamination cycle. There were additional factors that proved to be necessary prior to determine any specific parameters, such as sporicidal agent selection and behavior (adherence to surfaces and temperature changes impact).

Table 4 shows the cycle parameters for the different methodology test used during this study. Note that the same parameters were used for all of the test cycles with the exception of the set quantity of H_2O_2 during the Conditioning and Biodecontamination phase. These parameters were tested and equipment commissioning. Therefore it was recommended by the manufacturer and accepted by the client to fix the rest of the parameters to simplify the cycle development to assure a successful cycle design and to determine the cycle efficacy.

Based upon results of the methodology test performed during this study the final adjustment of the Total quantity of H_2O_2 during the Conditioning phase was adjusted to 1500g, see Table 4. This quantity will assure to meet the objective of reducing microorganism to zero (0) positives of BI's (6 log) placed within the isolator system.

The evaluation of the aeration time showed results of less than 1 ppm, the sample interval was decreased from thirty minutes to one sample every five minutes until three consecutive readings of <1ppm were obtained. The test determined that 2 hours were required to bring the hydrogen peroxide levels to <1ppm. This parameter includes a 30 min safety margin.

The attention to details and adjustments performed during the cycle development phase test execution ensured a smooth cycle validation.

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