The Effect of the Vaporized Hydrogen Peroxide Residues in a Trypticase Soy Agar Plates for a New Barrier Isolated Syringe Filling Machine

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Abstract *— This study has been performed to evaluate the feasibility and adequacy of the Trypticase Soy Agar (TSA) plates in promoting growth after being exposed to Vapor Phase Hydrogen Peroxide (VPHP) decontamination cycle. This project has been successfully completed in two phases. Data obtained from Phase 1 demonstrated that the growth promotion properties of the Becton & Dickinson Sterile Triple bagged of TSA plates were not impacted after being exposed to up to three consecutive decontamination cycles in the new syringe filling line isolator at the established decontamination cycle parameters. The results obtained from Phase 2 as well demonstrates no impact in the growth promotion properties after being exposed to a decontamination cycle, unpacked and the individual plates exposed to 1 ppm VPHP residues up to two (2) hours in a simulated manufacturing process when using the Quality Control Work Center isolator.*

Key Terms *— isolator decontamination cycles, Trypticase Soy Agar plates, vapour phase hydrogen peroxide.*

INTRODUCTION

The biotechnology pharmaceutical industry is one of the most important industries in the world discovering, developing, manufacturing and marketing human therapeutics based on advances in cellular and molecular biology. For almost 40 years, Puerto Rico has been a home to a majority of the major pharmaceutical and medical device manufacturers [4].

A new biotechnology pharmaceutical facility is constructing in Puerto Rico. The principal operation will be the aseptic filling of manufacturing products. The aseptic filling manufacturing process requires the continuous monitoring of the environmental conditions. As part of this requirement, viable particles are monitored during the process. The Trypticase SOY Agar (TSA) media plate, which is a general purpose medium used to support the growth of a variety of microorganisms such as bacteria and fungi is used for this intention.

The new filling line is completely integrated to an isolator; therefore supporting areas are designed to operate based on barrier isolation technology. This equipment is fully integrated to the hydrogen peroxide (H_2O_2) decontamination system, designed to use Vapor Phase Hydrogen Peroxide (VPHP) as decontamination agent.

For the decontamination cycle, the isolator chamber will be load with all required equipment and materials for the manufacturing process. It is expected to include TSA media packs as part of the isolator load, since they will be used for the environmental monitoring of the viable particles during the manufacturing process.

RESEARCH DESCRIPTION

This design project assesses the effect of the VPHP in the growth promotion properties of the Becton and Dickinson (BD) TSA media plates after been exposed to decontamination cycles. This investigation will provide data when the BD Sterile Tripple Bagged Pack of TSA media plates are exposed to decontamination cycles; and the unpacked plates to VPHP residues of 1 ppm for a period of two (2) hours.

This research will make a significant contribution for the isolator manufacturing process oriented to achieve an operational excellence system.

- The BD TSA plates are validated and currently used for the environmental monitoring of the existing manufacturing operations. No additional tests and costs required related to the introduction of additional plates to the manufacturing process while maintaining the reliability and product quality.
- Provide flexibility for the manufacturing operations to replace the TSA plate after two (2) hours of exposure in comparison with the actual replacement period of one (1) hour. This represents an improvement in terms of manpower utilization efficiency.
- For the isolators, it is commonly use TSA plates with Pyruvate for the environmental monitoring since it is effective to support growth promotions after being exposed to higher VPHP residues; however this project will provide documented evidence of the effect of the VPHP residues in the growth promotion properties of the TSA media plates after being exposed to decontamination cycles.
- The execution of this project provides the opportunity to implement TSA plates for the environmental monitoring in the isolator filling processes.

BACKGROUND

The new manufacturing facility intended to formulate and fill liquid biological drug products. This facility will be designed, validated, and operated to meet Current Good Manufacturing Practices (cGMP's). It serve as a key component for the overall risk mitigation strategy to ensure supply to patients and is designed to align with the current industrial standard for aseptic manufacturing, to meet an increasingly stringent regulatory environment, to improve production efficiency, and to lower manufacturing cost.

Manufacturing Process Overview

The manufacturing process of the new syringe filling line will be a continuous fill system process using three (3) production suites: the De-bagging area, the Filling room and the Tub labeling area. These productions areas provide the required conditions and equipment to produce aseptic manufacturing of syringe products. The syringe filling process includes the following equipment:

- Tub De-Bagger system
- E-Beam system
- De-Lid De-Liner system
- Filler machine integrated to an isolator system
- Tub identification system

As part of the manufacturing process, the bagged tubs are manually feed into the system conveyor where, the de-bagging process can be performed manually or automatically. Then, the tubs are transferred to the E-beam system for external decontamination. Once the tubs complete the e-beam stage, they are automatically transferred to the filling area. In this area, the decontaminated tubs pass into the isolator's De-lid/De-lining station. Syringes are then filled via filling needles and each filled barrel is fitted with a plunger stopper. The nest and tub, with the closed filled syringes are transferred from the filling isolator to the filling machine out feed conveyor. Tubs continue through to the tub labeling room for proper identification. Figure 1 provides an illustration of the manufacturing overview.

The syringe filling area is fully integrated to an isolator chamber which creates and maintains a Grade A (ISO 5) environmental conditions. This chamber covers and protects the filling equipment where the product and sterilized components are exposed to environment. The utilization of the isolator technology in combination with the E-Beam tunnel is predominately used in Europe [7]. This combination is also being utilized in the United States and it will be implemented as part of the new syringe filling manufacturing process.

Isolator Technology

The isolator consists of a main chamber full ceiling with sealed High Efficiency Particulate Air (HEPA) filters 99.99% efficient to provide an air quality that complies with Class A or ISO 5.

The isolator will used vaporized hydrogen peroxide as a chemical decontamination agent. The decontamination process using VPHP has been well studied and the data demonstrated that this method is effective to kill or inactivate pathogens microorganisms. There is evidence of the efficacy of room fumigation with vaporized hydrogen peroxide in decontamination of viable *Mycobaterium tuberculosi*, which cause a tuberculosis disease [2]. Also, it is demonstrated that virulent *Yersinis pestis* is inactivated in polymers, steel and glass surfaces when exposed to VPHP without observable physical damage to the test materials [5].

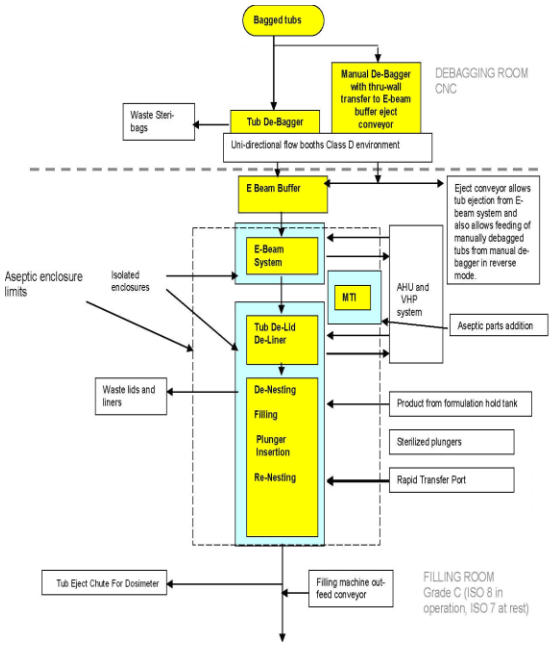

Figure 1
Syringe Manufacturing Process Overview

The surface decontamination of the new syringe filling line will be performed using hydrogen peroxide (H_2O_2) which is integrated into the isolator as a SIS 700 system. In the H_2O_2 surface decontamination, the overall bacterial reduction is obtained from the release of gaseous H_2O_2 and the effect of the lethal dose over time [6]. The decontamination process using vapor hydrogen peroxide (VPHP) consists of the following phases:

1. Pre-conditioning – the initial conditions required for the decontamination are created inside the chamber. This includes relative humidity (RH) and temperature (T) conditions.
2. Conditioning – the required H_2O_2 gaseous dose is generated in the chamber to obtain the microbial reduction.
3. Decontamination – the effective H_2O_2 gaseous dose is kept stable in the chamber for the period of time required for the microorganism reduction.
4. Aeration – the required residual amount of H_2O_2 in the chamber is achieved through the fresh air purging.

While the decontamination process is running, chamber parameters are monitored and VPHP concentration controls the process. After the exposure phase is completed, aeration steps will ensure that the VPHP is removed from the chamber. The aeration phase ensures an adequate environment for the manufacturing products. Since some of the syringe manufacturing products may adversely react when in contact to VPHP, it is required that the aeration phase removes the decontamination agent to reach levels of 0.03 ppm inside the isolator chamber.

The chemical activity of the decontaminating gases and residuals was identified as an obstacle that had to be overcome with the isolator implementation [1]. To mitigate this risk, a decontamination cycle has been developed to assure the reduction of the VPHP residuals inside the chamber to 0.03 ppm levels. However, one of the challenges for the isolator manufacturing process is the introduction of TSA media plates to monitor the environmental conditions inside the chamber since they could be sensitive to higher concentration of VPHP residues.

The air monitoring process is a GMP requirement for United States and Europe regulatory agencies. This monitoring could be difficult when using TSA plates after being exposed to VPHP decontamination agent. Previous studies were performed to evaluate the effect of VPHP residuals in the TSA media plates. An investigation demonstrated that concentrations of 3 ppm

inhibited the microorganisms growth in the TSA media plates [8]. Another investigation demonstrated that VPHP residual as low as 0.3 ppm can inhibit the growth of microorganisms after concentration on agar, rendering microbiological air monitoring difficulty [3]. Studies adding 1% pyruvate to the TSA plates proves that this formulation will tolerate as much as 8ppm residual VPHP ensuring growth promotion and avoiding any false negative results when air sampling. Both investigations provide valuable information related to the effect of the VPHP in the growth promotion properties of the TSA media plates. However, some limitations are found:

- The data presented does not include details of the decontamination cycle and parameters.
- Data presented does not include the exposure period of the TSA exposure to the VPHP residues
- No data presented related to the introduction of the plates into the isolator.

In summary, the utilization of the isolator technology in the pharmaceutical industry has been well studied and documented. The biotechnology industry is currently moving from the conventional clean rooms to the isolator technology. The decontamination process using VPHP has been demonstrated to be effective to inactivate pathogens microorganisms, however higher residues on the isolator chamber can have an impact to sensitive materials like TSA media plates.

METHODOLOGY

The design project will be performed in two phases. The first one consists to evaluate the feasibility and adequacy of the TSA media in promoting growth after being exposed in a triple bagged pack to decontamination cycles. This phase will be performed using the syringe filling line which is installed in a temporarily location at the existing facility since the new building is under construction. This location provides all the utilities and conditions required for the normal operation of the syringe filling equipment.

The second phase consists to expose a triple bagged pack of TSA to a decontamination cycle and then, unpacked the TSA media plates and exposed them to 1ppm VPHP residues for 2 hours. This phase will be executed in a manufacturing simulated environment using the Quality Control (QC) Work Center isolator since the syringe filling line will be installed into its final destination. This equipment provides all the conditions required for this test. The approach of this phase is to expose the TSA media plates to a worst case scenario of 1 ppm VPHP residues since the syringe manufacturing operations allows maximum VPHP residues of 0.03 ppm during the process.

Phase 1: TSA Triple Pack Exposure to Decontamination Cycles

A total of six (6) TSA packs (triple bags pack) will be included as part of the isolator load. Table 1 provides a summary of the isolator hydrogen peroxide dosage for the decontamination cycle. The decontamination cycle will be executed with the following parameters:

1. Pre-conditioning Phase– T: 25 ˚C, RH 20%
2. Conditioning Phase– Time dosing 180 sec
3. Aeration- To reduce the residual amount of H_2O_2 to 0.03 ppm or lower
   a. Aeration 1: 2,700 sec
   b. Aeration 2: 12,600 sec

Table 1
Syringe Isolator Hydrogen Peroxide Dosage

Description	SIS 700 Pump 01	SIS 700 Pump 02
Main Dosage	250g	250g
Re-dosing	25g	25g
No. of dosages	8	8
Pump 1 speed	62%	58%
Pump 2 speed	61%	64%

A Picarro Hydrogen Peroxide Gas Analyzer will be connected to the isolator to confirm that the VPHP residues inside the chamber reach the value of 0.03 ppm at the end of the aeration phase of the decontamination cycle. The Picarro Gas Analyzer is a real time, trace gas monitor capable of measuring H_2O_2 with parts-per-billion (ppb) sensitivity. It is a

stand alone instrument that works independently of the isolator decontamination cycle. This Gas Analyzer will be connected to the isolator during the aeration phase.

TSA Triple Bagged Pack Decontamination Cycle Exposure Strategy

Three (3) decontamination cycles will be performed. The TSA media triple bagged packs will be exposed to three (3) decontamination cycles as follows:

- Two (2) TSA packs will be exposed to one (1) decontamination cycle
- Two (2) TSA packs will be exposed to two (2) decontamination cycles
- Two (2) TSA packs will be exposed to three (3) decontamination cycles

Once the decontamination cycle is completed and the Picarro Gas analyzer reading detect a value of at least 0.03 ppm, two (2) TSA packs will be removed from the isolator. The TSA packs will be removed from the isolator through the out feed conveyor. The samples will be delivered to the Quality Control (QC) Microbiology laboratory to be tested for Growth Promotions following the established analytical method.

Phase 2: Unpacked TSA Plate Exposure to VPHP residues

This phase will be executed in a simulated manufacturing environment using the isolator located at the QC Work Center. The decontamination cycle of this isolator is currently validated and it is used for laboratory tests. In this phase the TSA triple bagged pack will be exposed to a decontamination cycle; then the TSA plates will be unpacked and exposed to 1 ppm VPHP residues. The VPHP residues will be measured using a drager tube system.

One (1) TSA triple bagged pack (10 plates) will be included as part of the QC Work Center isolator load for the decontamination cycle. The decontamination cycle parameters are included as follows:

- Dehumidity Time: 30 min at 2.3 mg/L
- Conditioning/Decontamination Time: 1 hour, 25 minutes
- Injection Rate: 2.8g/min
- Aeration Time: 30 minutes

Once the VPHP levels inside the chamber reach a value of 1 ppm, two (2) TSA media plates will be unpacked and exposed to the isolator environment using the Sterilizable Microbial Atrium (SMA) air sampling system. These plates will be exposed for two (2) hours. The air sampling equipment inside the isolator allows exposing two plates at the same time; therefore, the two TSA plates will be replaced every two hours. When the TSA plates are exposed to the required time, they will be labeled and tested for Growth Promotions following the established analytical method.

Growth Promotion Test

In the QC Microbiology laboratory, the TSA media plates will be located in a laminar flow hood (Class A). TSA media plates exposed to VPHP residues and the control samples will be inoculated. Table 2 indicated the microorganisms to be tested for growth promotion test.

Table 2
Microorganisms for Growth Promotion Test

Microorganism Name	Identification
Pseudomonas aeruginosa	ATTC 9027
Staphylococcus aureus	ATTC 6538
Bacillus subtilis	ATTC 6633
Candida albicans	ATTC 10231
Aspergillus brasiliencis	ATTC 16404
Bacillus (spp or flexus)	field isolated specie
Penicillium citrinum	field isolated specie

The TSA media plates will be located at the 30-35°C incubator for three (3) days. After that time period, the TSA plates will be verified to confirm the growth promotion. The results obtained will be documented in the approved laboratory form, evaluated and compared with the control samples.

RESULTS

The two phases of this project were sucessfully completed. The execution of the project run was initiated on 11/24/10 and completed on 05/04/11.

Phase 1: TSA Triple Pack Exposure to Decontamination Cycles

As part of the initial execution activities, on 11/24/10 all required materials for the test run were verified and properly documented. The filling line equipment including the isolator internal and external walls were cleaned using lint free wipes wet with Isopropyl Alcohol (IPA) 70%. The twenty four (24) isolator gloves were visually inspected to confirm its integrity and ports were hand sanitized with IPA 70%. At the end of the cleaning process, all isolators doors were closed. The isolator remains idle until 11/26/10.

On 11/26/10, the isolator doors were opened to load the chamber with all required materials for the test run execution. The isolator load included the following components:

- Eight (8) triple bag packs of TSA -The test run required the use of six(6) packs for testing. Two additional packs were included as spares.
- Three (3) empty syringe tubs
- Two (2) scissors
- One (1) forcep
- One (1) basket
- Two (2) tri-clamps ¾”
- Three (3) tri-clamps of 1 ½”
- One (1) tweezer

The components loaded in the isolator chamber for this test run represents a partial load as compared to the projected load for normal manufacturing operations. It was decided to include only the minimum required materials as a worst case scenario to stress the TSA media bagged plates with the VPHP during the decontamination cycle.

The eight (8) TSA packs were tie wrapped to the isolator tube located at the top of the chamber. They were distributed to covered the entire isolator chamber. The other components were located in the basket and around the filler machine. Figure 2 includes the specific location for the loaded components inside the isolator. When the loading activity was completed, the isolator doors were closed.

The Picarro gas analyzer fitting was connected to the isolator port and the valve to collect the gas samples from the isolator chamber was manually closed. Finally, the twenty four (24) glove stretchers were installed to complete the set up process.

Three (3) consecutive isolator decontamination cycles were sucessfully completed and no critical alarms were reported. Table 3 provides a summary of the cycles.

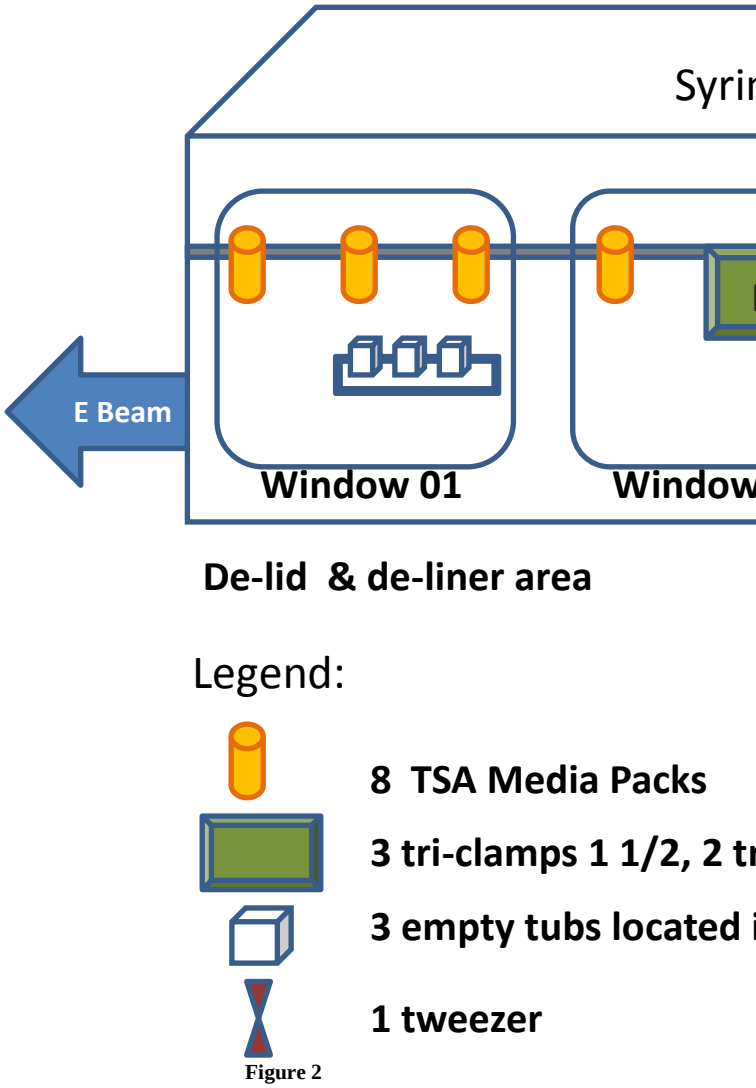

Figure 2
Syringe Isolator Load

Table 3
Syringe Isolator Decontamination Cycle Summary

Description	Cycle No. 1	Cycle No. 2	Cycle No. 3

Cycle start date/time	11-26-10/ 07:02	11-26-10/ 13:46	11-26-10/ 20:36
Conditioning phase start time	07:26	13:59	20:48
Conditioning phase end time	08:27	14:59	21:49
Aeration 1 started	08:28	15:00	21:49
Aeration 2 started	09:13	15:45	22:34
VPHP Aeration phase 2	0.096ppm	0.132ppm	0.152ppm
Manual Aeration 3 started date/time	11-26-10/ 12:46	11-26-10/ 19:19	11-27-10/ 02:07
Manual Aeration 3 end date/time	11-26-10/ 13:09	11-26-10/ 19:58	11-27-10/ 02:53
VPHP residues at the end Aeration phase 3	0.033 ppm	0.033ppm	0.033 ppm

TSA plates were exposed to decontamination cycles as summarized below:

- Twenty (20) TSA media plates (2 packs) exposed to one (1) VPHP cycle
- Twenty (20) TSA media plates (2 packs) exposed to two (2) VPHP cycles
- Twenty (20) TSA media plates (2 packs) exposed to three (3) VPHP cycles.

A total of eight (8) TSA media packs (10 plates each) were initially included as part of the isolator load. When the first decontamination cycle was completed, and the VPHP concentration inside the isolator reached at least 0.03ppm, two (2) TSA packs were removed from the isolator.

In order to avoid unnecessary manipulations with the glove stretchers, the TSA plate packs were removed from the isolator according to glove locations represented in the Figure 3. All TSA packs were removed from the isolator by the outfeed conveyor, labeled and delivered to the Quality Control (QC) laboratory for testing.

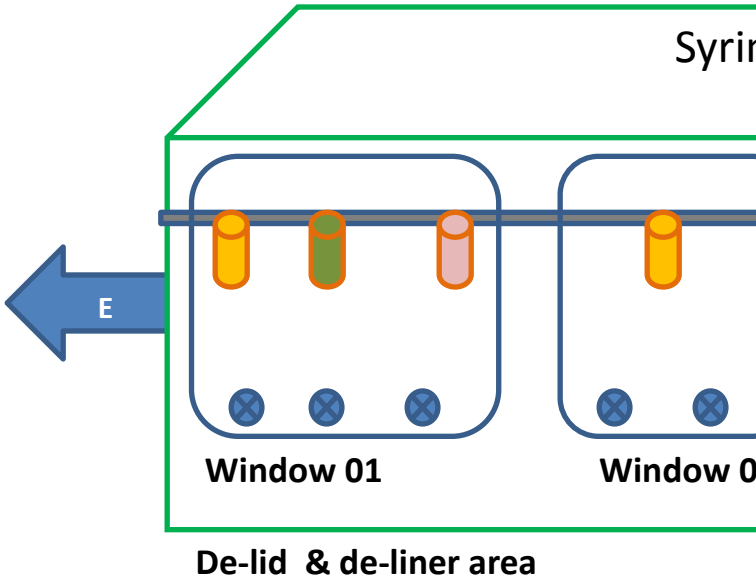

Figure 3

TSA Pack Removal Illustration

The TSA packs were removed from the isolator as described below:

- After Cycle # 1: Packs removed from location 1 and 4.
- After Cycle # 2: Packs removed from location 2 and 5.
- After Cycle # 3: Packs removed from location 3, 6, 7 and 8.

At the end of the third cycle, the two (2) additional TSA packs included as part of the isolator load were delivered to QC lab for appropriate disposal.

The TSA media plates were tested for Growth Promotion per laboratory analytical method. A total of seven (7) microorganisms including American Type Culture Collection (ATTC) and isolated microorganisms were challenged as part of the test method.

These microorganisms were inoculated in the TSA media plates identified as control samples and test run samples. Duplicate plates from the control samples and the test runs were inoculated using the spread plate technique. It consists of taken specific volume of the microorganism, inoculating the media plate and spread across the agar surface using a sterile bent glass rod or equivalent.

All samples were incubated at 30-35 ºC for three (3) days. The growth promotion results obtained for the three runs are within the acceptable control limits established by the laboratory analytical method.

The data collected after Cycle #3 represents the worst case scenario since the TSA media packs were subjected to three consecutive decontamination cycles. The results obtained showed that the microorganisms tested in these plates rendered growth as a normal condition. Figure 4 graphically represents the growth promotions results obtained.

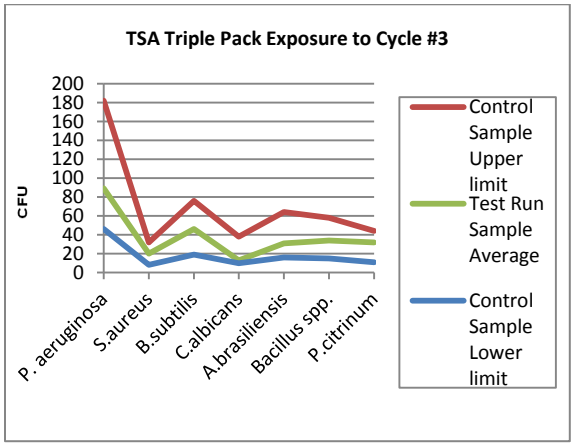

Figure 4
Growth Promotion Results after Decontamination Cycle #3

Phase 2: Unpacked TSA Plate Exposure to VPHP residues

This phase was executed under a manufacturing simulated scenario using the QC Work Center Isolator located at the Microbiology laboratory. A comparison of the new syringe manufacturing isolator and the QC Work Center isolator was performed. It was observed that the main difference between the syringe line and the QC isolator is the capacity or area. The syring line isolator area is 515 f^3 and the QC Work Center isolator is 95 f^3. Based on the size, the syringe line isolator includes two (2) VPHP stations and the QC Work Center isolator only one (1). A summary of the Syringe line isolator and QC Work Center isolator comparison is included in Table 4.

In terms of the decontamination cyle, both isolators use a 35% of Hydrogen Peroxide. Each cycle parameter has been designed per the specific requirements of the functional area.

Table 4
Syringe Line Isolator Vs. QC Work Center Isolator

Description	Syringe Line Isolator	QC Work Center Isolator
Isolator area	515 f^3	95 f^3
Hydrogen Peroxide Concentration	35%	35%
VPHP stations	2	1
Total VPHP per cycle	900g	238g
VPHP per isolator area (1)	1.74g/ f^3	2.5g/ f^3
Condition/Decontamination Time	1 hr	1 hr, 25min
Main Dosage	250 g	N/A
Time dosing	180 sec	N/A
Re-dosing	25g	N/A
Number of dosages	8	N/A
VPHP Injection Rate	N/A	2.8g/min
Aeration 1	45 min	30 min
Aeration 2	210 min	N/A
Manual aeration	Mouseholes opened	N/A
VPHP concentration at the end of cycle	0.03ppm	1 ppm

The conditioning phase time period for the syringe line is one (1) hour and the QC Work Center is one (1) hour and twenty five (25) minutes. During this phase, the syringe line isolator requires a total of 900g of hydrogen peroxide and the QC isolator 238g per cycle. The use of the hydrogen peroxide represents a VPHP per area of 1.74 g/ f^3 for the syringe line and 2.50 g/ f^3 for the QC Work Center isolator. The VPHP per area was calculated using the following formula:

$$x = a/b \quad (1)$$

Where a= hydrogen peroxide required per cycle, b= isolator area and x= VPHP per area.

Based on the evaluation of the decontamination cycles, it was observed that the decontamination cycle of the QC Work Center isolator represents a "worst case" scenario for the TSA exposure of the VPHP residues, since it requires a highest quantity of hydrogen peroxide per area. In addition, at the end of the cycle, the VPHP residues allowed for the QC Work Center process is 1ppm in comparison of 0.03 ppm for the syringe manuafcturing process.

QC Work Center Isolator Decontamination Cycle

A total of three (3) decontamination cycles were completed and no alarm was reported. Table 5 provides a summary of the cycles. During the aeration phase, the VPHP levels were measured using the drager tube system (tubes drager short term detector). The Drager tube® is a glass vial filled with a chemical that reacts to a specific chemical of family of chemicals. This tube was connected to the isolator port where a calibrated 100mL of air is drawn through the tube with the Drager bellows pump. In this case, the chemical inside the tube reacts at the range from 0.1 to 3 ppm of hydrogen peroxide gas and its color change from white to brown.

Once the VPHP levels inside the chamber reach a value of 1 ppm, two (2) TSA media plates were unpacked and exposed to the isolator environment using the air sampling Sterilizable Microbial Atrium (SMA) system.

A total of fourteen (14) TSA plates were exposed to VPHP residues of 1 ppm at the QC Work Center isolator environment. Each plate was exposed for two (2) hours. Once the TSA plates were exposed to the required time, they were labeled and tested for Growth Promotions following the established analytical method.

Growth Promotion Results after 2 hours of VPHP exposure

The growth promotion results obtained for the three runs are within the acceptable control limits established by the laboratory analytical method.

The data presented on Figures 5 demonstrates that the growth promotion properties of the TSA media plates after being exposed to up to two (2) hours of 1 ppm VPHP residues at the QC Work center isolator are within the acceptable limits established by the analytical method.

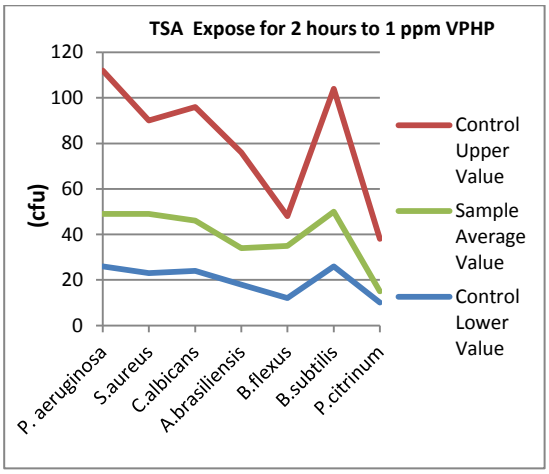

Figure 5
Growth Promotion Results

Table 5
QC Work Center Isolator Decontamination Cycle Summary

Description	Cycle No. 1	Cycle No. 2	Cycle No. 3
Cycle start date/time	03/16/11 06:27	03/17/11 08:42	05/04/11 06:52
Conditioning phase start time	07:13	09:30	08:39
Conditioning phase end time	08:38	10:55	09:04
Aeration started	08:38	10:55	09:04
Aeration end	09:08	11:25	09:34
VPHP measured by drager tube	1 ppm	1 ppm	1 ppm

CONCLUSION

This study has been performed to evaluate the feasibility and adequacy of the Trypticase Soy Agar plates in promoting growth after being exposed to Vapor Phase Hydrogen Peroxide decontamination cycles. The data obtained provides evidence of the effect of the VPHP on the BD Trypticase Soy Agar (TSA) plates.

The objective of this project was to provide an alternative of using TSA plates, currently used for the existing manufacturing process, to monitor the environmental conditions of the new barrier isolated syringe filling line manufacturing operations. This alternative represents a contribution in terms of the costs reduction related to the introduction of additional materials to the manufacturing process while maintaining the product quality. This project was successfully executed in two (2) phases:

- Phase 1: TSA triple packs exposed to up to three (3) consecutive decontamination cycles using the new filling line isolator
  - Two (2) packs exposed to one decontamination cycle
  - Two (2) packs exposed to two decontamination cycles
  - Two (2) packs exposed to three decontamination cycles
- Phase 2: TSA triple pack exposed to one (1) decontamination cycle and unpacked the plates exposed to 1 ppm VPHP residues for two (2) hours at a simulated manufacturing process using the QC Work Center isolator.

The TSA media effectiveness after being exposed to decontamination cycles using VPHP was challenged through a Growth Promotion test. Seven (7) microorganisms including American Type Culture Collection (ATTC) and isolated microorganisms were challenged: *Pseudomonas aeruginosa, Candida albicans, Aspergillus brasiliencis, Staphylococcus aereus, Basillus (spp and flexus), Penicillium citrinum* and *Bacillus subtillis*.

All data and results gathered during the execution of the study were evaluated and properly documented. The growth promotion data observed during the two (2) phases studied showed that the microorganisms tested in the TSA plates rendered growth as a normal condition. Based on the results obtained, it can be concluded that:

- The growth promotion properties of the BD Sterile Triple bagged of Trypticase Soy Agar (TSA) plates were not impacted after being exposed to up to three consecutive decontamination cycles in the new syringe filling line isolator, when the decontamination cycle is set with the hydrogen peroxide dosage included in Table 6 and the following parameters:
  - Pre-Conditioning T 25°C, RH 20%
  - Condition Phase Time dosing: 180 sec
  - Aeration 1: 2,700 sec
  - Aeration 2: 12,600 sec
  - Manual Aeration 3: (Mousehole 1-infeed, Mousehole 2-outfeed and flaps opened, Transition Laminar Air Flow (LAF3) turned on).

Table 6
Hydrogen Peroxide Dosage

Description	SIS 700 Pump 01	SIS 700 Pump 02
Main Dosage:	250g	250g
Re-dosing:	25g	25g
Number of dosages	8	8
Pump 1 speed	62%	58%
Pump 2 speed	61%	64%

- The growth promotion properties of the BD Sterile Triple bagged of Trypticase Soy Agar (TSA) plates were not impacted after being exposed to a decontamination cycle, unpacked and exposed to 1 ppm VPHP residues up to two (2) hours in a simulated manufacturing process when using the Quality Control Work Center isolator.

The successfully results obtained as part of this study, demonstrates that the growth promotion properties of the TSA media plates are not adversely impacted after being exposed to VPHP decontamination agent, therefore, it can be considered for the environmental monitoring of the manufacturing process in the new barrier isolated syringe filling line.

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