Use of Experimental Design for the Characterization of a Depyrogenation Machine

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Abstract *— Depyrogenation tunnel are used in pharmaceutical filling lines to sterilize glass containers before they are aseptically filled. These tunnels use unidirectional hot air at temperatures up to 350°C and can be used to sterilize and depyrogenated glass vials and glass containers. The experimental design presented in this project describes the performance qualification activities process in the dry heat depyrogenation tunnel at worst case conditions ensuring the integrity of the HEPA filters. The parameters considered were: belt speed, temperature and endotoxin reduction. The parameters identified after analyzing the measurement are to be improved by applying DMAIC methodology from Six Sigma.*

Key Terms *— DMAIC, experimental design, performance qualification, HEPA filters.*

INTRODUCTION

For a biotechnology manufacturing facility, cleaning certainly is a critical process. 21CFR Part 211.94 states: "Drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use." A pyrogen is defined as a fever-producing agent. Pyrogens are substances that cause febrile reactions when sufficient amounts enter the circulatory system. Bacterial endotoxin is the most significant pyrogen because of its potency and ubiquity [1].

When new equipment is introduced to a manufacturing facility its cleaning process is validated. Validation of cleaning procedures is necessary for the following reasons: it is a customer and regulatory requirement and also assures from an internal and compliance point of view the quality of the process. The validation exercises confirm that the established cleaning methods are consistent and effective in the removal of pyrogenics from the equipments after being used in the products manufacturing process. Endotoxins are poisonous substances that are produced in bacteria, and continue to exist after the bacteria has been destroyed. Therefore, a sterile surface may still retain dangerous endotoxins [2].

The manufacture of modern pharmaceuticals is a complex process involving highly technical personnel, complex equipment, sophisticated facilities and complicated processes. For that reason the Food and Drug Administration (FDA) is responsible for regulating and supervising the safety of foods, drugs, vaccines, biological medical products and cosmetics. In June 1906, President Theodore Roosevelt signed into law the Food and Drug Act, also known as the "Wiley Act" after its chief advocate. The Act prohibited, under penalty of seizure of goods, the interstate transport of food which had been "adulterated", with that term referring to the addition of fillers of reduced "quality or strength", coloring to conceal "damage or inferiority," formulation with additives "injurious to health," or the use of "filthy, decomposed, or putrid" substances. The act applied similar penalties to the interstate marketing of "adulterated" drugs, in which the "standard of strength, quality, or purity" of the active ingredient was not either stated clearly on the label or listed in the United States Pharmacopoeia or the National Formulary. The goal of the regulatory agencies is to ensure quality control, quality assurance for all aspects of the pharmaceutical processes. Pharmaceutical, bio-medical device and even food preparation industries are concerned about the cleanliness-physical, chemical and especially biological of their

products. These industries have materials that need regular careful cleaning and some have requirements for validating such cleaning, demonstrating that required levels of cleanliness has been met. They want to prove the efficacy of their cleaning methods. For example, " Cleaning and validation of cleaning are among the most critical issues facing producers of recombinant DNA protein products, monoclonal antibodies (MAbs) and oligonucleotide therapeutics," according to [Adner and Sofer (1994)].

Dry Heat Tunnels for Depyrogenation Vials

Validation of dry-heat tunnels is demonstrated by both temperature measurements and inactivation of bacterial endotoxins (depyrogenation). Similar studies to dry-heat ovens are performed, i.e., empty tunnel temperature distribution and loaded tunnel endotoxin challenge. The temperature variation within the sterilization/depyrogenation zone may be greater than seen in an oven. Higher temperatures are usually selected - ranging from 270°C - 350°C, due to the shorter exposure time required and the greater temperature variation. When the exposure time is longer the temperature selected is ≤ 370°C to obtain operational conditions based on endotoxin reduction. Points to consider for physical measurements include:

- Belt Speed - determines the exposure time.
- Temperature- determines the time required for inactivation.

The air used in the depyrogenation process must be filtered by leak free HEPA grade air filters. Depyrogenation tunnels and continuous ovens are continuous systems that are used to reduce the amount of endotoxin to an acceptable level on glass or metal vials or other process containers and accessories. Depyrogenation ovens are batch systems used to reduce the amount of endotoxin to an acceptable level on glass or metal vials or other process containers and accessories.

The items being subjected to the depyrogenation process must remain at the specified temperature for the specified time period for the process to be successful. Reduced heat-up and cool down periods as well as increased maximum temperature can increase the total throughput of the equipment.

Developing a process validation strategy early in clinical development is critical to the execution of a successful validation program because process validation is more than just running three consecutive batches under protocol. The magnitude of activities leading to the qualification batches requires resources and expertise that far exceed those in place for routine development and production.

The DMAIC Methododology

The Six Sigma DMAIC process methodology is a system that brings measurable and significant improvement to existing processes that are falling below specifications. The DMAIC methodology can be used when a product or process is in existence at your company but is not meeting customer specification or is otherwise not performing adequately [3].

DMAIC is an acronym for five interconnected phases:

- Define the project goals and deliverables for both internal and external customers
- Measure the process to determine current performance
- Analyze and determine the root cause(s) of the defects
- Improve the process by eliminating defects
- Control future process performance

Define

In the Define phase, the Six Sigma project team identifies a project for improvement based on business objectives and the needs and requirements of the customers. Six Sigma is about "solving a problem with an unknown solution." To unearth the solution, the problem needs to first be defined in concrete measurable terms. Six Sigma is focused on finding out directly from customers what their idea of quality is, and how well the current process meets that standard.

Measure

In the Measure phase, the team begins with the proper metrics. Critical measures that are necessary to evaluate the success of the project are identified and determined. The initial capability and stability of the project is determined in order to establish a measurement baseline. Valid and reliable metrics to monitor the progress of the project are established during the Measure phase; input, process, and output indicators are identified. Once the project has a clear definition with a clear measurable set of indicators, the process is studied to determine the Key Process Steps and an operational plan defined to measure the indicators.

Analyze

Through the Analyze phase, the team can determine the causes of the problem that needs improvement and how to eliminate the gap between existing performance and the desired level of performance. This involves discovering why defects are generated by identifying the key variables that are most likely to create process variation. As the Six Sigma team moves through the Analyze stage and subsequent Improve stage of the process they will discover various process improvement scenarios and determine which has the best net benefit impact to the company. A common error people make when they discuss Six Sigma is thinking that the DMAIC process takes too long to achieve improvements. This is far from the truth. Quick improvements are often achieved early in the project and frequently already implemented by the time the team reaches the Analyze phase. If the team has not already identified major improvements, then the breakthrough often results from careful process analysis with data. Six Sigma analysis techniques are valuable tools to uncover more difficult solutions. A variety of methods are used to identify potential root causes, narrow down the possibilities, and confirm the relationship between the suspected causes and the performance of the process.

Improve

The Improve phase is where the process transitions into solutions. Critical inputs have been verified and optimized toward nailing down the problem causes. Once problem causes are determined in the Analyze phase, the team finds, evaluates through testing, and selects creative new improvement solutions. The team identifies and quantifies what will happen if needed improvements are not made and what will happen if the improvements take too long. This develops a cost/benefit analysis. More often than not simple process experimentation and simulation bring the team big gains in this step. Also at the Improve stage, the team develops an implementation plan with a change management approach that will assist the organization in implementing and adapting to the solutions and the changes that will result from them.

Control

Success in the Control phase depends upon how well the team did in the previous four phases. The keys are a solid monitoring plan with proper change management methods that identify key stakeholders. Lessons learned are now implemented and tools are put in place to ensure that the key variables remain within the acceptable ranges over time so that process improvement gains are maintained. The team develops a project hand off process, reaction plans, and training materials to guarantee performance and long-term project savings. Documenting the project is very important so that the new procedures and lessons learned are maintained and provide concrete examples for the organization. At the close of the Control phase, ownership and knowledge is transferred to the process owner and process team tasked with the responsibilities. Finally, the team identifies what the next steps are for future Six Sigma process improvement opportunities by identifying replication and standardizations opportunities and plans.

METHODOLOGY

DMAIC methodology was used to determine the causes of defect to improve and comply with specifications. It consisted in determining the current manufacturing process conditions and provides improvements. It is identifying as a traditional define, measure, analyze, improve and control DMAIC Six Sigma strategy.

DMAIC: Define Phase

Depyrogenation and sterilization processes are used to eliminate viable matter and reduce the amount of endotoxin on vials or other containers used in pharmaceutical processing and distribution. These processes utilize dry heat at a prescribed temperature and duration. It is up to the pharmaceutical manufacturer to decide what cleaning, sterilization, and depyrogenation is appropriate for their given process. The main objective is apply DMAIC tools during the qualification activities of depyrogenation machine by identifying the worst case conditions in terms of temperature and belt speed ensuring the integrity of the HEPA filters after manufacturing process use.

The exercise consisted in design specifications to meet the following Test Run purposes:

- Temperature above the HEPA filters was maintained ≤ 370°C to ensure the integrity of the filters over time.
- Characterize the tunnel at different heater temperatures and belt speeds to obtain optimum operational conditions based on endotoxin reduction.

METHOD FOR THE EVALUATION OF PARAMETERS IN DEPYROGENATION PROCESSES

The laminar flow dry heat depyrogenation tunnel, Despatch Tunnel, installed in manufacturing room is used to depyrogenize glass vials, where they are exposed to a flow of unidirectional heated air (Figure 1). It is composed of the following zones:

Preheat Zone – This zone prevents hot airfrom entering the washer and the washroom in order to avoid damaging plastic parts or causing personnel injury. Washed vials enter against the positive air pressure of the inlet air shower, where they are heated by forced convection.

Hot Zone – In the hot zone, the vials are brought to a depyrogenating temperature (250 °C) and maintained at this temperature long enough to ensure complete depyrogenation. Air is drawn through electric heating coils and then distributed in a laminar flow through high temperature (400 °C rated) HEPA filters, and onto the vials and through the conveyor belt. The hot zone heater temperature can be varied depending upon process requirements. To prolong the life of the HEPA filters, the hot zone temperature above the HEPA filters should not exceed 370 °C.

Cool Zone - As vials exit the depyrogenation zone, they are cooled to about 25°C. Both sides of the Cool Zone are sealed off with gates. The exit gate prevents excessive sterile air from flowing into the tunnel. Another gate prevents larger amounts of air from entering the Hot Zone.

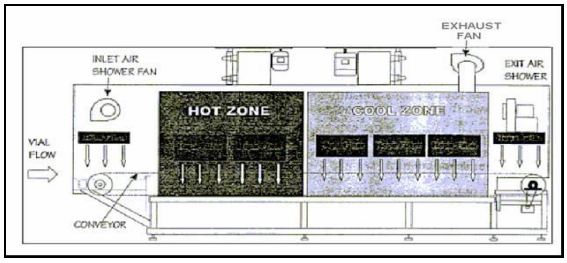

Figure 1
DepyrogenationTunnel

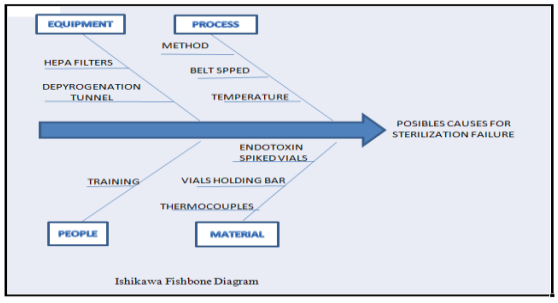

Figure 2
Fishbone Diagram

The Fishbone Diagram was utilized to visualize possible causes for sterilization Failures (Figure 2). In the Fishbone Diagram the different factors that could affect the process were evaluated.

DMAIC: Measure Phase- Experimental Method

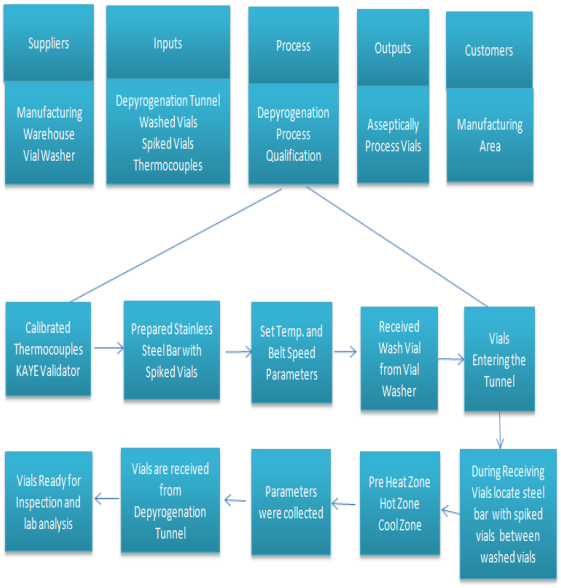

Figure 3
SIPOC Diagram

The project consisted of the following tests: Loaded Tunnel Characterization Study:(Figure 3). Having selected the initial heater temperature set point from the above study, a set of loaded tunnel runs were performed to gather data for determination of optimum operational conditions and validation "worst case" parameters, based on endotoxin reduction (3-log reduction minimum. Test runs for heater temperature set points from 295 ºC to 320 ºC and belt speed set points from 90 mm/min to 110 mm/min were performed during this study for 5 cc vial sizes. The cooling zone temperature set point was maintained at 25 ºC in all the runs. Data for belt speed, temperature from hot zone sensors and temperature above the HEPA filters were collected, along with results for endotoxin log reduction to determine optimum operational and "worst case" parameters. In addition, data for penetration temperature, FH, differential pressures and cooling zone temperature were also collected to support analysis and obtain a complete tunnel performance profile. Data for belt speed, temperature from hot zone sensors and temperature above the HEPA filters were collected, along with results for endotoxin log reduction to determine optimum operational and "worst case" parameters.

Endotoxin population reduction was verified for a total of ninety (90) vials in different runs during the characterization exercise. Each spiked vials in a run contains a minimum of twelve thousand (12,000) endotoxin units. Vials were collected at the tunnel exit and analyzed by a qualified microbiology laboratory analyst.

DMAIC: Analize Phase Evaluation of the Tunnel Parameters for "Worst Case" and Operational Parameters Runs

The tests reported here are aimed at evaluating the effect of the main parameters that affect the reduction of endotoxins in vials; belt speed and temperatures.(See table 1).

Table 1
Factors vs. Response

Factors	Responses
Temperatures (295,300,320)	Endotoxin Results
Belt Speeds (90, 100, 110)	

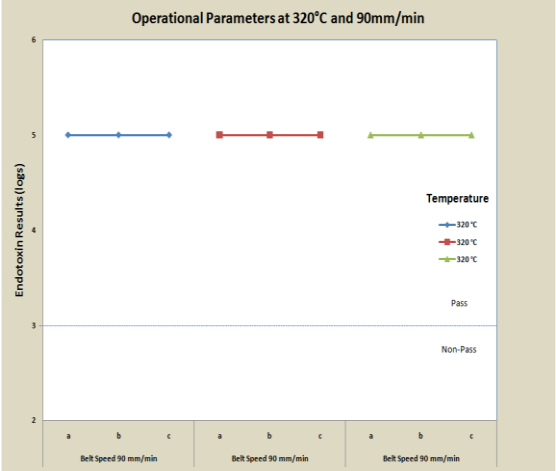

Figure 4
Main Effects Plot for Operational Parameters

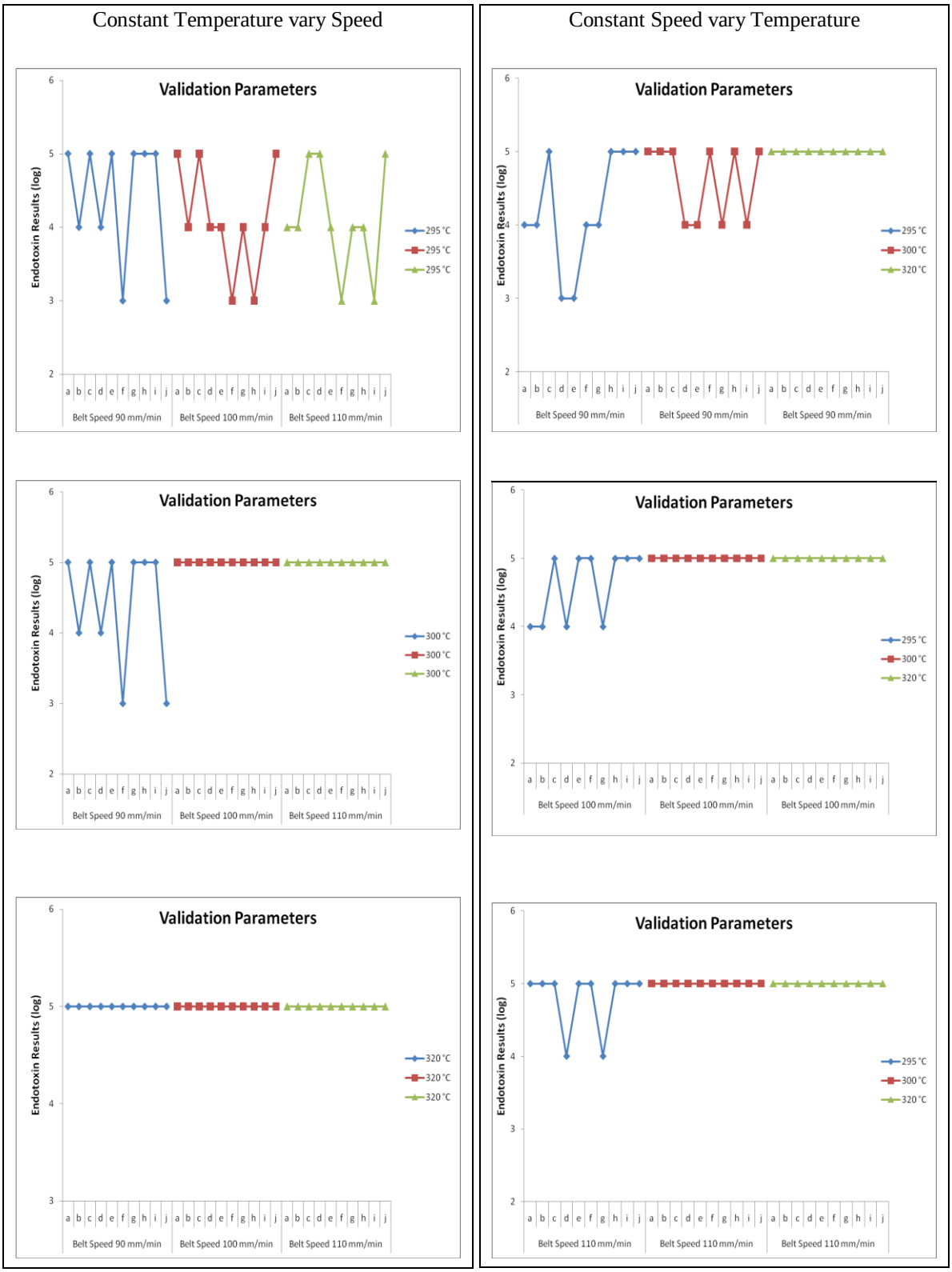

Figure 5
Validation Parameters Comparisson

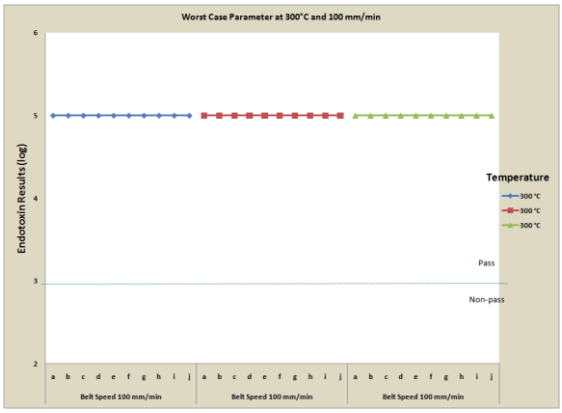

Figure 6
Main Effects Plot for Worst Case Results

Table 2
Analysis Results

Key Parameter	Validation ("Worst Case") Parameters After	Normal Operating Parameter Before
Hot Zone Heater Temperature Setpoint	300 ºC	320 ºC
Belt Speed	100 mm/min	90 mm/min
Gate Height (mm)	50	

The results from the application of statistical analysis are useful when compared the results obtained from worst case parameters.

The results of the statistical analysis lead to the following: the method developed to examine the influence of the different parameters on depyrogenation gives results in agreement with previous experimentation reported. (Table 2).

DMAIC: Improve Phase

The data shows that the tunnel hot zone temperature sensors were in the range of 291 – 311°C (296 ± 5 °C) and a belt speed range of 98 - 107 mm/minutes (102.5 ± 4.5 mm/minutes) for the "Worst Case" parameters runs, while 311 – 331°C (321 ± 10°C) and 86 – 92 mm/minutes (89 ± 3 mm/minutes) for the Operational parameters runs. Under these conditions the endotoxin reduction level obtained was 5 logs for the "Worst Case" Parameters after and Operational parameters before. (Figures 4, 5 and 6).

DMAIC: Control Phase

Based on the documented evidence and results from this study, it is recommended to proceed with the formal performance qualification activities scheduled for this unit at the following "Worst Case" operating limits using endotoxin challenge only:

- Tunnel Hot Zone heater temperature setpoint not less than 300°C
- Conveyor Belt Speed setpoint not less than 100 mm/minutes.

Key operating parameters for this tunnel are depyrogenation temperature setpoint and belt speed, for which the following values are recommended. The following recommendation is based on the characterization results:

The table below includes the temperature range found from the data of hot zone (HZ) sensors for the "Worst Case" and Operational parameters runs considered by this characterization with the recommended operational temperature limits. (Table 3).

Table 3
Recommended Operational Parameters

Hot Zone (HZ) Sensor	"Worst Case" Temperature Range, ºC	Temperature Range (Recommended Operational Temperature Limits), ºC
HZ Entrance	291 – 299 (295 ± 4 °C)	311 – 319 °C (315 ± 4 °C)
HZ Center	298 – 302 (300 ± 2 °C)	318 – 322 °C (320 ± 2 °C)
HZ Exit	301 - 311 °C (306 ± 5 °C)	318 - 331 °C (324.5 ± 6.5 °C)

CONCLUSION

The material presented in this project concerned depyrogenation vials, which is considered to be one of the most critical processes of a pharmaceutical plant. Since the main purpose of requiring cleaned equipment is to maintain the product integrity by preventing contamination and adulteration, the validation of any critical cleaning process related to equipment of finished drugs and active pharmaceutical ingredients is an expectation of the regulatory agencies.

The statistical analysis of the results shows that, the parameters proposed for the depyrogenation machine are significant and that all the parameters examined were critical to maintain the HEPA filters integrity.

The results of the statistical analysis lead to the following:

- The method developed to examine the influence of the various parameters provided results in agreement with previous characterization.
- The interactions between these variables (temperature and belt speed) are proven to be equally important to obtain good results for endotoxin log reduction and for maintain filters integrity and good depyrogenation. The optimal levels corresponding to these factors were adequately selected and recommended.

FUTURE WORK

It is recommended that future research be conducted in other depyrogenation machines to ensure the integrity of HEPA filters. The use of Six Sigma (DMAIC) should be one of continuous usage to measure process performance, identification of improvement opportunities and correction; not only in the direct manufacturing area, but also on those areas that support the direct manufacturing of a product.

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