Optimization of Bioburden Samples Processing in the Microbiology Laboratory Using Six Sigma

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Abstract *— This article presents the use of the Lean Six Sigma tool to identify factors affecting the processing of samples that require Bioburden testing in the Microbiology Laboratory. The objective of this article was to implement and design a system in which samples could be processed in the shortest time. The data was collected using LIMS System and behavior was evaluated by watching the analyst during test execution. Then, a statistics test helps to compare and determine if the new design fulfills the objectives of the research. Our findings suggest that the Bioburden tests of water samples and final products are more manageable than those of In-process samples. However, we conclude that the processing times of the samples were significantly reduced.*

Key Terms *— Bioburden, Lean Six Sigma, Microbiology, Quality Control.*

INTRODUCTION

The research is related to optimizing the processing of samples in the Bioburden test in the Microbiology Laboratory. The Bioburden test helps determine the number of bacteria or fungi associated with a particular product or sample before it is sterilized or continues with the manufacturing process [1]. The filtered samples are also related to the raw material, assembly processes, manufacturing environment, water, lubricants, and cleaning processes. It has vital importance due to the stability and quality of the product could be determined based on the results obtained in this test. For this reason, this research seeks to implement a system to process Bioburden samples in the shortest possible time. Lost time is considered a waste according to the Six Sigma philosophy. Lean Six Sigma is a process improvement methodology used to increase product quality based on waste reduction [2]. Any bioburden control strategy is related to three essential elements:

- Assessment of bioburden control needs at different steps of the production process.
- Confirmation of expected performance under process conditions.
- Maintain continuous process monitoring to confirm bioburden control during each production cycle.

The research aims to implement and design a system by which the samples can be processed in a shorter period, avoiding the loss of time during Bioburden testing and manufacturing product processes.

The research implemented will help to understand the Bioburden assay, having the benefit of optimizing sample processing in a shorter period. By identifying and applying the improvements, certain stages of production will be unaffected by not performing the Bioburden test on time. The results of this research will also contribute to developing a structured system allowing the processing of the most significant number of samples in the shortest possible time. The first step will be to train the employees in the Six Sigma philosophy to understand the background of this model proposed for sample processing.

REVIEW OF LITERATURE

The pharmaceutical industry is identified as one of the most prominent and influential business sectors worldwide. Pharmacopeia is the official legal tool that guarantees product quality, which includes all the quality standards for formulations, active ingredients, and drug manufacturing.

Additionally, this regulation has all the microbiological analyses that must be carried out on pharmaceutical products, raw materials, water, air, equipment, and packaging material. [2]

Quality control is increasingly important in the manufacture of biopharmaceutical products. It can be defined as complying with customer requirements or federal and international regulations. Several rules are established during manufacturing, including laboratory tests, environmental monitoring, personnel monitoring, and other controls. Different techniques and tools are applied to improve the quality of the process by reducing its variability to comply with the highest quality of the product [3]. Regarding microbiological controls, all rooms in controlled environments must be continuously monitored. These controls include microbial control of environments and microbiological control of surfaces and equipment. It is to detect any significant changes in the bacterial presence. Also, it prevents the release of potentially contaminated batches of products that do not meet the established standards and serves as a tool to measure the effectiveness of the sanitary measures adopted in the company. These microbiological monitoring must be carried out in compliance with government requirements and regulatory agencies. Including, as a minimum, qualified culture media capable of detecting the presence of bacteria, molds, and yeasts, requirements for the flow of personnel, equipment, and materials, and procedures indicating sampling locations. These requirements and regulations are outlined in the U.S. Pharmacopeia section "Microbiological Control and Monitoring of Aseptic Processing Environments." [2].

Microbiology supports in the pharmaceutical industry is an essential resource because it plays a crucial role in the production and analysis processes that guarantee the quality of pharmaceutical products. Part of the tests performed in the Microbiology laboratory includes the Bioburden test. Bioburden refers to the microbial levels or content on a particular sample. These samples include water, raw materials, in-process products, and others. This test can determine if any upstream/downstream steps or additives solutions are compromised. Bioburden testing uses membrane filtration or the pour plate method [1].

Lean Six Sigma establishes that to increase product quality, the waste associated with the process must be eliminated or reduced. One of these wastes is the loss of time and motion. [4]. Lean Six Sigma has five steps that must be performed to accomplish its implementation. These steps are called DMAIC (Define, Measure, Analyze, Improve, and Control) [5]. Several research results using the Six Sigma method proved that the success in the implementation is based on the consistency of DMAIC. They are providing positive results in problem-solving.

METHODOLOGY

The project was initiated by performing a literature review of previous studies to evaluate how to implement the DMAIC methodology of Lean Six Sigma. This research helped to define the problem that needs to be solved. After this research, the employees were trained in this philosophy.

After the problem was defined, the next step was to collect Bioburden historical data through Laboratory Information Management System (LIMS) to get information about processes that were to be improved, check if there was enough data, documentation of the current situation, and perform comparative tests. Bioburden samples were evaluated to determine the time it took to process each since it was received in the laboratory until it was incubated. The samples evaluated in this project were filtered using the membrane filtration method. These are filtered through a membrane with a pore size of 0.45um. Also, the materials and media location were considered a factor that influenced the performance of the test. For this reason, Bioburden materials and media were rearranged to help minimize the time during sample processing (Figure 2). Using the new laboratory

design, data were collected again using LIMS Software.

To analyze the effectiveness of the implementation, a statistical test was performed to study the data and establish a comparison. The data were compared to see if the changes helped reduce sample processing time. After this, it was possible to determine three main groups of factors: work organization, method, and man.

The main task to analyze the improvement was to perform a fishbone diagram to determine the effect of the implemented changes. This diagram will help identify if additional modification is required to optimize the process.

After implementing the changes, an additional step will be performed to control and check if any other factors influence the process. A control plan must be executed to evaluate and examine the process periodically. If any non-conformance is detected, action must be started to solve the further problem. After implementing the changes, an additional step will be performed to control and check if any other factors influence the process. A control plan must be executed to evaluate and examine the process periodically. If any non-conformance is detected, action must be started to solve the further problem.

RESULTS AND DISCUSSION

The results obtained in this project followed the improvement established by the DMAIC model.

Defining and Measuring

Three tests were selected to perform the Bioburden processing samples optimization assessment (Water, Final products, and in-process products assays). After the problem was defined, the next step was to collect historical data and make a comparison between this data and the data obtained after implementing the new design. To establish the historical data, an initial assessment was carried out to determine the time it took to process the samples from when they were delivered to the laboratory until they were incubated. In addition, to evaluate the time, technicians were observed to know how they performed the test. Figure 1 shows the steps to follow when a sample is received in the laboratory until it is processed using the Bioburden test.

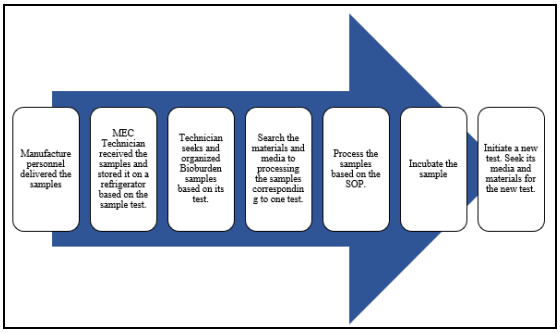


Figure 1
Process Map

Also, infrastructure and materials/media location were evaluated to determine the changes and new organization. It helps to establish the changes and the organization that must be carried out to optimize the sample processing. Figure 2 shows the initial location of the materials and media used in the bioburden test only.

Figure 2
Laboratory Facilities Organization
Diagram legend: (1) Biosafety Cabinet 1, (2) Chemical Cabinet, (3) Biosafety Cabinet 2, (4) Buffers cabinet, (5) Funnels cabinet, (6) Computer, (7) Environmental media, (8) In-process samples & (9) Computer. It also includes the Incubators and Cold Room (where Bioburden material and media were stored).

After performing the initial assessment, it was found that one of the reasons for the long time spent processing the samples was that the technician needed to incubate the samples as soon as the test was completed. This cause had yet to be considered initially, but it turned out to be

meritorious to include it in the training on the Six Sigma methodology. Also, it was found that although the technicians use the same procedure, the number of samples they filter will depend on who executes it. It could represent a delay if the number of samples received increases.

Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Water	23	8	86.2	14.1	67.7	15.0	45.0	60.0	106.0	278.0
Final Product	16	15	135.0	35.5	141.9	16.0	33.3	50.0	233.0	433.0
In Process Products	30	1	105.0	12.2	66.8	30.0	54.5	89.5	120.0	300.0

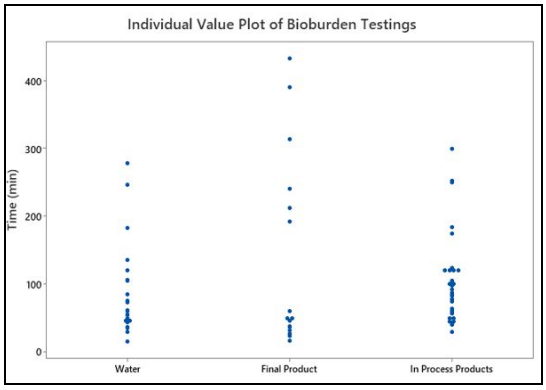


Figure 3
Descriptive Statistics and Individual Value Plot of Initial Assessment Based on Bioburden Tests

In accordance with the objectives, it was measured the time it took to complete each of the Bioburden tests for one week. This time was evaluated from the start of the test until the samples were incubated. The time from the receipt of the samples until the test's beginning was not considered. Due to a lack of trained personnel, the samples were stored for a long time. Figure 3 shows the individual plot value and descriptive statistics obtained after the initial assessment. In the graph, it was observed a significant dispersion of the values about the processing time in the different tests. After evaluating the data obtained, the personnel were trained. The training was related to the fundamentals of the Six Sigma methodology and its advantages. Also, the results were presented by measuring the processing time and the possible causes of the delay in the tests. It helped determine possible solutions and how to prevent it. To prevent materials from running out and having to look for the warehouse. At the beginning or the end of the shift, the materials should be evaluated to determine if there is enough to process the samples received. In addition, the changes that would be made in the laboratory were explained so that everyone knew the new location of the materials.

Analyze

In this phase, the results were analyzed on the new model implemented in the Microbiology Laboratory (see Figure 4).

Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Water	23	8	61.22	4.98	23.90	15.00	45.00	58.00	76.00	106.00
Final Product	16	15	68.1	10.7	42.8	16.0	33.3	50.0	113.5	140.0
In Process Products	60	1	83.40	5.40	41.82	30.00	50.00	76.50	100.00	215.00

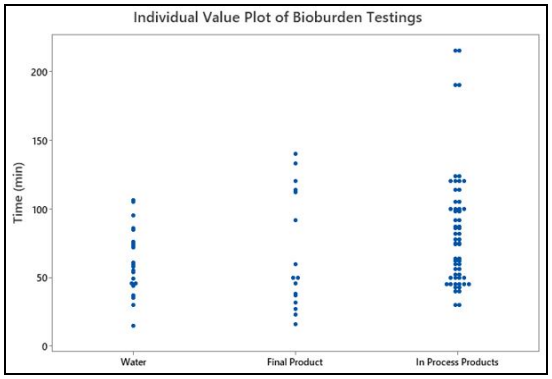


Figure 4
Descriptive Statistics and Individual Value Plot After New Design Implementation

After having made the rearrangements to the materials used in the Bioburden test, the time elapsed in each of the tests was re-evaluated. For this, the technicians were observed to corroborate that they applied what was discussed in the training. In this way, doubts can be clarified. As a result, an improvement in sample processing time was achieved. Each of the changes helped minimize the time lost in the search for materials when a new Bioburden test was performed. In the latest results obtained in the graph after implementing the new organization model, it was possible to observe a significant difference related to the testing of water and final products. However, the In-process product test remains the same results. Not all technicians feel confident processing more than one product at a time. So far, this does not represent a delay in sample processing if the samples are incubated after the test. However, if production were to increase, this could present a problem. Compared to the previous data, there was a decrease in the standard

deviation, indicating that the changes made were successful for this test.

The hypothesis test study is based on a two-tailed paired T-test, as an alternative hypothesis was established about the difference between the two means. The P and T values were used to determine whether the null or alternative hypothesis was accepted or rejected. In this case, the P value obtained was 0.047 using a significance level of 95% (see Figure 5). When comparing this data with alpha, it was observed that the P value is less than the significance level (alpha = 0.05). For this reason, it can be concluded that the null hypothesis is rejected, and the alternative hypothesis is accepted. It implies a significant difference between the means of both samples. After comparing the significance level, the T-value was evaluated. The T value obtained was 2.10 and based on the amount of data per sample, which was 23, the degrees of freedom were calculated. For both samples, the degree of freedom was 22. Having this data, the T-test distribution table was used to determine the critical value for a two-tailed T-test at a significance level of 0.05. As a result, the critical value was 2,074. When comparing the obtained value with the critical value, it can be concluded that the null hypothesis was rejected since the experimental value was greater than the critical value. Therefore, there is a significant difference between the two means.

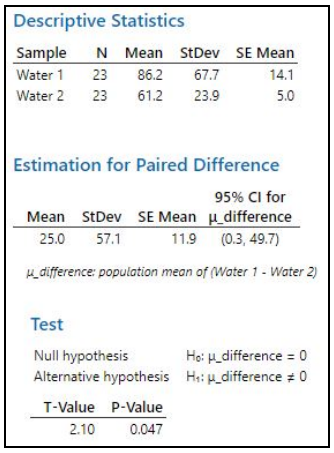


Figure 5

Descriptive Statistics and Hypothesis Test for Water Samples Using Minitab Statistical Software

However, the hypothesis test using the final product data significantly improved (see Figure 6). It can be observed when comparing both standard deviations of the samples (before and after). Initially, the standard deviation was 141.9, and after implementing the changes, it was reduced to 42.8. This means that the data is three times less dispersed than initially. When evaluating the mean of both samples, they are not close. The current average is almost half of the initial value. A paired T-test with two tails was used to perform the hypothesis test. Since the objective was to corroborate if the difference between the means is equal to or different from zero, the P values and the T value were analyzed. The P-value was 0.021 with a significance level of 95% (alpha=0.05). When comparing the value obtained with alpha, it was observed that it is lower than the significance level. It implies that the null hypothesis is rejected, and the alternative hypothesis is accepted. The difference between the means is different from zero.

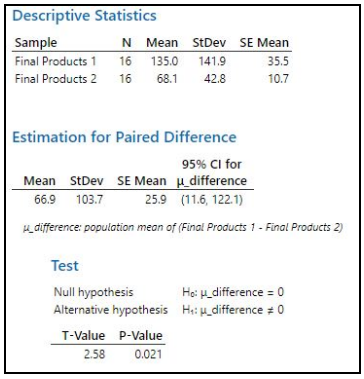


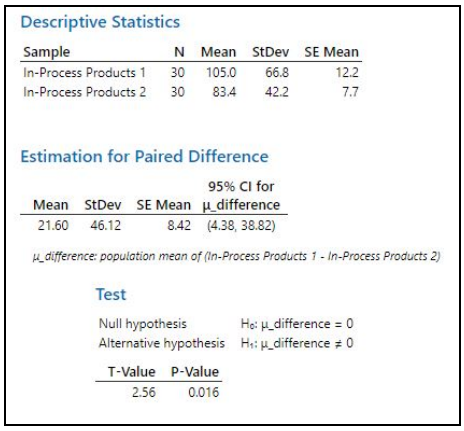
Figure 6

Hypothesis Test for Final Products Using Minitab Statistical Software

On the other hand, the T-value obtained was 2.58. The degree of freedom for this sample set is 15. A distribution table of critical T values with a significance level of 95% got a theoretical T value of 2.131. When comparing the experimental T-value with the critical T-value, it was observed that the obtained value is greater than the critical value. So, it can be concluded that the null hypothesis is rejected, and the alternative hypothesis is accepted. At the T-value obtained not to be close to zero, it is

more likely that there is a significant difference between the means. Like the P-value, this indicates a significant difference between the two means of the final products.

Finally, the hypothesis test was also performed on samples related to In-process products (see Figure 7). Before starting, the data obtained in the descriptive statistics were evaluated. Unlike the previous data in the other samples, for this test, there was no significant difference in the standard deviation and mean of both samples. Both the standard deviation and the means remain very close. It is because this test is sometimes run in different ways. It is one of the limitations since the procedure to follow for these tests is more flexible than the others. It allows technicians to process samples individually, making processing time longer. After evaluating the descriptive statistical data, the P-value and T-value were examined using a significance level of 95%. The P value was 0.016, and when compared to alpha, it equals 0.05. The value obtained is lower than the significance level, so it is concluded that the null hypothesis is rejected, and the alternative hypothesis is accepted. It implies that there is a significant difference in the means.



Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
In-Process Products 1	30	105.0	66.8	12.2
In-Process Products 2	30	83.4	42.2	7.7

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
21.60	46.12	8.42	(4.38, 38.82)

μ_difference: population mean of (In-Process Products 1 - In-Process Products 2)

Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
2.56	0.016

Figure 7
Hypothesis Test for In-Process Products Using Minitab Statistical Software

On the other hand, the T-value was 2.56. The degree of freedom for this dataset was 29. The critical value was obtained using the degrees of freedom and a distribution table T. The critical value was 2,045. When compared with the value obtained, it was observed that this is greater than the critical value. So, it was concluded that the null hypothesis was rejected, and the alternative hypothesis was accepted. It indicates a significant difference in the means of both samples because the contrast of the means is not close to zero.

Improve

The results presented were done in collaboration with a peer at the laboratory. To be able to train the technicians on the Six Sigma methodology and to be able to get everyone to perform the test in the same way. After evaluating the results obtained in the hypothesis test, it was determined that as part of the training that will be provided to the employees, training should be included that reinforces the technician's confidence while handling the samples. During the meeting held with the peers, it was found that only some of the technicians felt safe when carrying out tests with products. It is considered a limitation. As a resolution for this problem, specific technicians should be exposed more to practice these skills and perfect their techniques during Bioburden tests. A fishbone diagram was made to evaluate the other causes mentioned during the meeting that affect this test. They considered the environment/facilities, personnel, equipment, and control (see Figure 8).

One of the evaluated causes was the staff, where it was mentioned that some needed to be more familiar with the procedures. For this, it was suggested to give refresher to the employees occasionally to prevent it. These refreshers can be provided through an online platform so that the employee can have them accessible when needed. Another reason was that the technicians at the time of execution had to make unnecessary movements. It could imply a waste of time.

For this reason, the reorganization of the materials and media was implemented to prevent this from happening. In addition, there needs to be more staff to carry out the tests at the most critical moments. It is not only due to the low number of technicians and the rise in manufacturing but only

some technicians are trained in specific tests. It is a limitation since if a stat sample arrives at the time of trained personnel, a technician must be called who is uncalled. Staff should have to cross-train to ease the workload. Another cause was the facilities or environment, as the materials needed to be organized by test. Due to the number of samples received, the equipment has been saturated with samples. It could be a limitation eventually. To avoid this, other equipment should be provided to organize the samples by test. Even though quality control laboratories have many restrictions and regulations, certain essential factors, such as follow-up on training, are not considered. It consists of the expiration of these and training all personnel. As part of the continuity controls that should be measured is the time it takes to process a sample. Factors like this could define how soon a manufacturing team can be used or the release of a batch. These causes and resolutions were considered when the new design was implemented. These are essential factors for the success of the model.

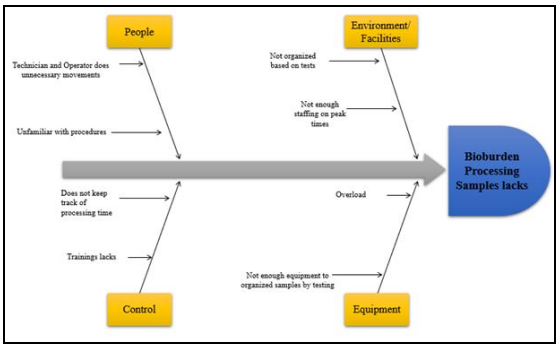


Figure 8
Fishbone Diagram

Figure 9 shows the improvement model implemented in the laboratory. Physically, there was no significant change related to the equipment and cabinets; only the chemical cabinet was transferred. The other equipment remained original; only the materials were reorganized so that they were more accessible to achieve the Bioburden testing (see Figure 9). The media used for the Bioburden test were transferred from the Cold Room to the laboratory. At the same time, the media for environmental monitoring were placed in the Cold Room. These are required once per shift, which is a minor change for environmental monitoring. Another of the recommendations that were made was to provide a refrigerator to store the samples since more than one that is in one is needed. It will require different approvals, so it is not contemplated to be implemented during this project.

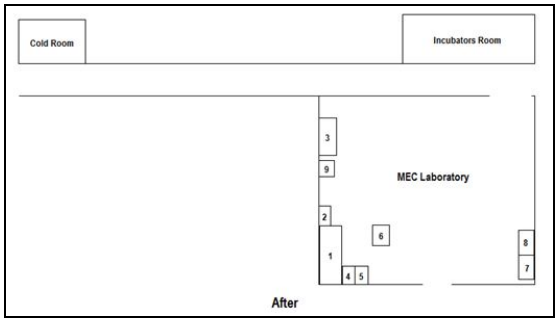


Figure 9
New Laboratory Facilities Organization
Diagram legend: (1) Biosafety Cabinet 1, (2) Chemical Cabinet, (3) Biosafety Cabinet 2, (4) Buffers cabinet, (5) Funnels cabinet, (6) Computer, (7) Bioburden media, (8) In-process samples & (9) Computer. It also includes an Incubator room and Cold Room (where Environmental material and media were moved).

Control

To sustain the changes made and suggested in this project, a periodic evaluation of the test will be executed. It is a way to maintain the implemented changes and to address causes of Bioburden tests that must be solved. It would help to run the test without any mishaps. As previously mentioned, more personnel should be trained, and both Biosafety Cabinets enabled at times when the Bioburden test is saturated with tests pending to be processed. It would also help decrease processing time. The Bioburden results are critical in some stages since they determine whether to continue to the next stage or require other tests. It is one of the reasons why sample processing in this was raised as a problem. Another issue that must be worked on is implementing a digital resource or platform that is accessible to clarify doubts if the technician must troubleshoot the samples. Also, so that the employee can fully understand the procedures, they

should be reviewed to verify that they are explicit and detailed. It would avoid or solve doubts when performing a test. Sometimes, when the procedures need to be clarified, it can lead to misinterpretations and, consequently, to mistakes that could have been avoided. Having this in mind, it will be helpful to develop a process flowchart related to the Bioburden tests, to visualize the different steps as established by the Standard Operating Procedure (SOP). This flowchart can be incorporated as an annex to the procedures. Also, if production increases, the changes should be re-evaluated to see if they continue to be effective.

CONCLUSION

According to the results, the objectives of this project were successfully fulfilled. The main goal was to implement a new design to process the samples in a shorter period. It was completed and accomplished when the new rearrangements were implemented. The changes made in the organization of the materials and media used in the Bioburden tests, a significant difference was observed in the two tests. Except for the In-process products test. It showed that small changes can be decisive when performing a test. Training the employees on the Six Sigma methodology was helpful for them to understand the reason for the changes. In addition, with the availability of personnel, solutions were obtained by exchanging ideas. Having listened to the ideas of my colleagues was helpful to be able to carry out the improvement part. The Fishbone Diagram was used to determine the root causes and the effects of each of them. These improvements have resulted not only in time reduction but also in cost reduction. To prevent a recurrence of the problem, it is crucial to continue educating and training the employees. In addition, it will periodically evaluate the execution of the Bioburden tests to verify that the changes made continue to be successful. It will help determine if there are any other areas of opportunity.

Lean Six Sigma was used throughout the project, as this methodology provides many benefits for the laboratory and manufacturing processes. Each stage in a manufacturing process is essential for the commercial product's quality. For this reason, every detail is considered, and the microbiological controls established by the regulatory agencies are periodically examined. If any of the quality controls fail, it could have serious consequences. Therefore, they are constantly reviewed for these to work successfully. In this project, based on the waste established by Lean Six Sigma, it was determined that the processing time of Bioburden samples was a factor that affected production, delaying specific processes and sometimes the relaying of batches. Lean Six Sigma, a process improvement methodology, helped optimize the efficiency of the test. That's why employees were educated about the method. Because not all employees knew about this methodology, the benefits that could be obtained if processes continue to be improved are cost reduction, improvement in the quality of the products, and more efficient processes, among others.

One of the main constraints of the project was the need for more staff training. Not all personnel master the Bioburden test, so this knowledge and skill should be reinforced so that sample processing is not affected. Also, there are few trained people; this is a problem at peak times. Because the workload falls on a limited group of people, to avoid this, it is contemplated to train most of the staff by making them have cross training. It would help alleviate the workload, preventing the team from feeling stressed and being able to make any human error. For this reason, the Bioburden test will continue to be evaluated to improve the sample processing until the before mentioned is not an impediment.

To maintain the continuous improvements of the Bioburden test, Lean Six Sigma techniques will continue to be used in Quality Control laboratories. It would help to improve other tests significantly. Initially, it was implemented using the Bioburden test, but a future goal is to implement this philosophy and methodology in the different

microbiological and analytical assays. It is imperative to continue monitoring and watching each process to corroborate that the implemented changes are effective. One of the most significant contributions of this project was to align laboratory personnel to a single modus operandi in the Bioburden test. It helps the test to be performed consistently and uniformly. It is making the samples process diligently. However, as a suggestion for people who may have doubts during the execution of the test, you should look for ways to make the procedure more visual. It could be done by adding a flowchart or pictogram that helps the technician determine where he is in case of doubts.

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