Improve Service and Efficiency of LAL Kinetic Chromogenic Method for a Combine Manufacturing Product

Sheila Reyes Donatiú
Master in Manufacturing Competitiveness
Advisor: Rafael Nieves, PharmD.
Industrial and Systems Engineering Department
Polytechnic Unversity of Puerto Rico

Abstract *– A new supplier is now validated according to the requirements on the USP <85> Bacterial Endotoxin Test, USP <161>, ANSI/AAMI ST72:2011. This validation is conducted in order to comply with manufacturing and customer needs. This company for over a hundred (100) years is dedicated to produce medical devices and advance the delivery of healthcare. Considering this information, we need to be persistent on our deliveries and to comply with this the validation of Supplier 2 is necessary. This validation become in a positive outcome when a time of test is reduced, then the documentation process is easier and faster. Also an economic yield is reflected.*

Key Words *— ANSI/AAMI, DMAIC, Inhibition/Screening/Enhancement, KQCL, LAL, LWR, MVD, Pyrogen, USP.*

INTRODUCTION

Delivering the best of their product must be the essence of every company. When working on a manufacturing environment you need to follow procedures. Actions that the operator perform, must be included or mentioned in these documents. The suppliers that provide to companies must be validated and be included in procedures according to regulations. It is important that procedures provide more than one supplier for the things the operator needs to assemble. The same thing occurs with laboratory tests. In order to perform the tests that confirm the excellent quality levels of products. For everything the test needs, procedures shall have more than one supplier. An opportunity is found in one of our procedures due to a distribution service inefficiency according to business needs. A new supplier shall be validated to eliminate waiting time, back orders and stopping the manufacturing line works. This promotes a better work efficiency and comply with our goal of advancing the delivery of healthcare.

RESEARCH DESCRIPTION

The LAL Kinetic Chromogenic method is used to test our products and raw materials. It is a quantitative assay for the detection of Gram Negative bacterial endotoxin. Individual analysis using LAL reagent water and glucashield as diluents for the lysate were performed. Initial Qualification assay on new Lysate of Supplier 2 was performed. Endotoxin standard preparation that was performed as instructed per manufacturer. The linearity of the standard curve demonstrated the Correlation Coefficient (r): between 0.980 to 1.000 and met USP regulation for initial standard qualification. Satisfactory results were obtained for the Standard Endotoxin concentration using Supplier 2 Control Standard Endotoxin (CSE).

Inhibition / Enhancement / Screening was conducted as per instructions stated in our procedures, the quantity of samples per lot will depend on the product /material select to be tested. As per our specified procedures, USP <161> and ANSI/AAMI ST72:2011 the selection of number of samples should be not less than three (3) and not more than ten (10) devices. Before conducting the Bacterial Endotoxin Test (BET), the test endotoxin limit for each Product must be determined. The test endotoxin limit defines the maximum allowable concentration of endotoxin that can be present in a product extract solution.

RESEARCH OBJECTIVES

The purpose of this study is to document strategy for qualification of Supplier 2 as an alternate reagent to be used for the detection of

bacterial endotoxin using the Limulus Amebocyte Lysate (LAL) Kinetic Chromogenic technique. This reagent will be used for LAL Kinetic Chromogenic for our Combined Manufacturing Product raw material, in-process water and final product. A new supplier, reagent and a different technique will be evaluated in order to avoid lost in sales.

RESEARCH CONTRIBUTION

The result of this KQCL test brings the manufacturing line the release they need along all their process, since the raw material arrives at our company. With the validation of Supplier 2 we have the opportunities to save on time usage and cost of testing materials as seen in Figure 1.

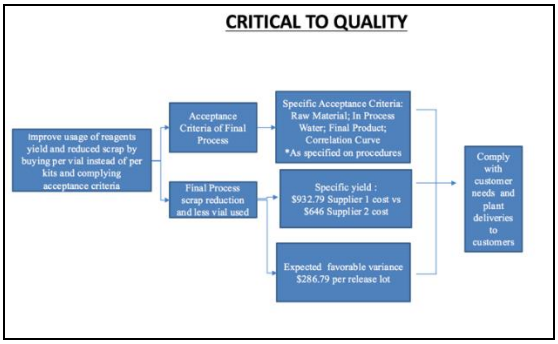

Figure 1
Critical to Quality Diagram

LITERATURE REVIEW

As a manufacturing company, we have various regulated agencies that govern us and some standards to comply. International Standarization Organization (ISO), specifically ISO 13485:2016 that specifies requirements for a quality management system where an organization needs to demonstrate its ability to provide medical devices and related services that consistently meet customer and applicable regulatory requirements [1]. In order to perform a good validation process in addition to ISO 13485:2016 we need to comply with the requirements of USP <85> Bacterial Endotoxin Test, USP <161> Transfusion and Infusion Assemblies and Similar Medical Devices, ANSI/AAMI ST72:2011 and our procedures. The USP <85> for a bacterial endotoxins test established that there are two types of techniques: Gel Clot Technique and the Chromogenic method (KQCL). The gel-clot technique, are based on gel formation, and the photometric technique and it is a qualitative assay. The chromogenic method, are based on the development of color after cleavage of a synthetic peptide-chromogen complex and it is a quantitative assay [2]. The USP <161> Transfusion and Infusion Assemblies and Similar Medical Devices established the requirements apply to sterile and nonpyrogenic assemblies. Also devices in contact directly or indirectly with the cardiovascular system, the lymphatic system, or cerebrospinal fluid. This regulation includes, but is not limited to: solution administration sets, extension sets, transfer sets, blood administration sets, intravenous catheters, implants extracorporeal oxygenator tubings and accesories, dialysers and dialysis tubing and accesories, heart valves, vascular grafts, intramuscular drug delivery catheters and transfussion and infussion assemblies [3]. The American National Standards Institute and the Asociation for the Advancement of Medical Instrumentation (ANSI/AAMI); ANSI/AAMI ST72:2011 is for bacterial endotoxins test methods, routine monitoring, and alternatives to batch testing [4]. Since our product labeling certifies that they are pyrogen free, we need to follow ANSI/AAMI ST72:2011. It is established by the regulation that a pyrogen is any substance that can induce fever [4]. To recognize any substance that can cause fever, the company could use LAL test, specifically Kinetic Quantitative Chromogenic assay (KQCL). This assay is usually extra sensitive to detect endotoxin limits of any product. To overcome interference while still allowing detection of endotoxin limits (EL), dilutions could be done on a product. It is important to establish that a product shall not be diluted beyond the point of being able to detect the endotoxin limit [5]. For any LAL assay the process shall have a maximum validation dilution (MVD). It is established by regulation the equation shall be used to calculate first de endotoxin limit:

$$EL = \frac{(K)(N)}{V} \quad (1)$$

Where:
K = Amount of endotoxin allowed per device;
N = Number of devices tested;
V = Total rinse/ soaking solution in combination of samples (ml).

Once the test endotoxin limit is calculated, a maximum validation dilution (MVD) shall be established by the following equation:

$$MVD = \frac{Test\ endotoxin\ limit}{\lambda} \quad (2)$$

Where:
Test endotoxin limit = EL equation;
λ = a provided value; the confirmed label claim sensitivity of the lysate reagent.

As part of this validation process we need to perform an inhibition/screening/enhancement test. This test is used to determine whether a particular BET sample contains factors that diminish its accuracy by introducing enhancement or inhibition into the test system. It is established on regulations that an inhibition/screening/enhancement is an anomaly in which a non-endotoxin related factor, usually attributable to a characteristic of the test sample, elicits a test reaction greater than the amount of endotoxin present.

To solve this supplier validation process, we shall use DMAIC; which is a six sigma methodology. A DMAIC is an acronym for the five phases which make up the process that is: define, measure, analyze, improve and control. On **define** we shall see the problem, improvement activity, the project goals, and the customer (internal and external) requirements. We shall **measure** process performance. **Analyze** the process to determine root causes of variation or performance defects. Then the process must be **improving** the performance by addressing and eliminating the root causes. Finally, **control** the improved process and future process performance [6]. The DMAIC methodology project an approach to quality improvement. An explicative diagram is shown on figure 2.

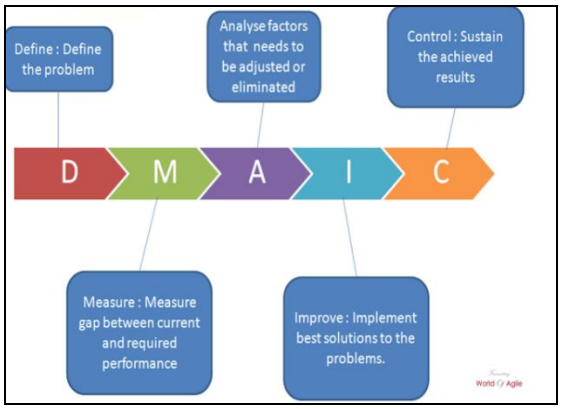

Figure 2
DMAIC Methodology Diagram [7]

METHODOLOGY

The research methodology DMAIC on this project consist on the following steps. First of all, the table 1 shown below the record time of this project is implemented because of the necessity of the company.

Table 1
Project Time per Activity

Activities	Time
Project time	3 months
Presentation	1 week
Define	1 week
Measure	2 weeks
Analyze	2 weeks
Improvement	2 weeks
Control	2 weeks
Final Review	2 weeks

The implementation of DMAIC, a six sigma methodology for KQCL to implement Supplier 1 to Supplier 2 was released on the following steps.

Define

Supplier distribution service inefficiency, delivery not on time based on business necessities, and supplier quality issues. Needs to look for an alternate supplier, plant was limited to only one approved supplier.

Measure

Individual analysis using LAL reagent water and Glucashield as diluents for the lysate were performed. Initial Qualification assay on new Lysate of Supplier 2 was performed. Endotoxin standard preparation that was performed as instructed per manufacturer that is shown on figure 3.

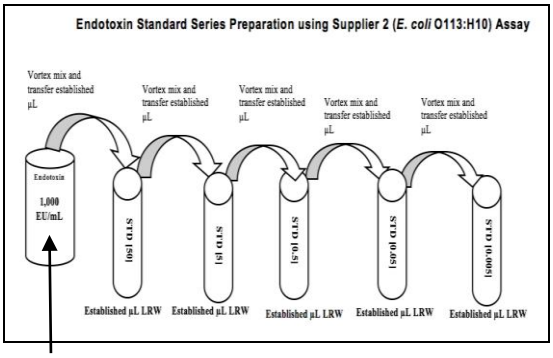

Figure 3

Dilution Series for Control Standard Endotoxin (CSE)

Inhibition / Enhancement / Screening was conducted as per instructions stated on our procedures. The quantity of samples per lot will depend on the product or material select to be tested. A table 2 below shown the lots selection.

Table 2

Lots Selection

Product	Quantity of Lots Required	Item Number	Lot Number
Raw material Sample	1	1X	X
In Process Water	1	1X	X
Final Product	1	1Y	Y

As per our procedures, USP <161> and ANSI/AAMI ST72:2011 the selection of number of samples should be not less than three (3) and not more than ten (10) devices. Before conducting the Bacterial Endotoxin Test (BET), the test endotoxin limit for each Product under the test must be determined; shown on table 3.

Table 3

Endotoxin Limit and Maximum Validation Dilution Table

Combined Manufacturing Products	Test Endotoxin limit	Maximum Validation Dilution
Raw material	Calculated per ISO Device or EU/mL	1:10 1:100 1:1,000 1: 10,000
In Water Process	Calculated per ISO Device or EU/mL	1:10 1:100 1:1,000
Final Product	Calculated per ISO Device or EU/mL	1:5 1:10 1:100

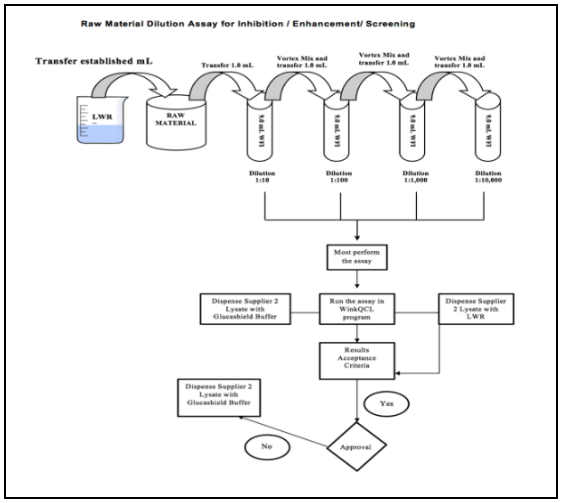

Figure 4

Raw Material Dilution Series for Inhibition, Screening and Enhancement

The test endotoxin limit defines the maximum allowable concentration of endotoxin that can be present in a product extract solution. For all dilution series the geometric mean raw material, in process water, and final product must confirm λ. If not, it may be necessary to dilute the sample further. Additives such as Glucashield buffer may be used according to manufacturer's instructions. Results for the inhibition/enhancement/screening will be invalid if the results of the negative control are positive or if the endotoxin/LRW control series do not confirm λ. A diagram of the dilutions for

Inhibition, Screening and Enhancement is shown below on Figures 4, 5, and 6 for Raw material, In Process water and Final Product respectively.

An established aliquot must be transferred from the initial vial shown as LWR to a raw material, then from vial to vial to perform the assay as see on Figure 4. If the assay fail, further actions shall be needed.

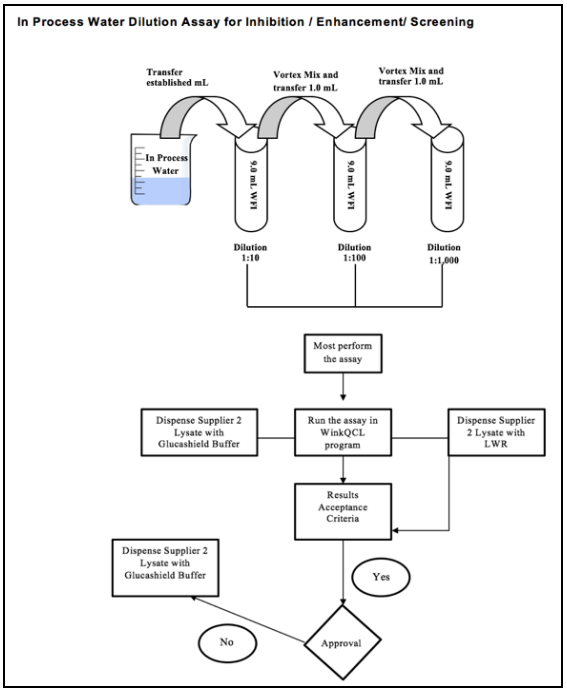

Figure 5
In Process Water Dilution Series for Inhibition, Screening and Enhancement

An established aliquot must be transferred from the initial vial shown as in Process Water to complete the dilution series, to perform the assay as see on Figure 5. If the assay fails, further actions shall be needed.

For the Final Product Dilution Series for Inhibition, Screening and Enhancement; an established aliquot must be transferred from the initial vial shown as LWR to complete the dilution series, to perform the assay as see on Figure 6. If the assay fails, further actions shall be needed.

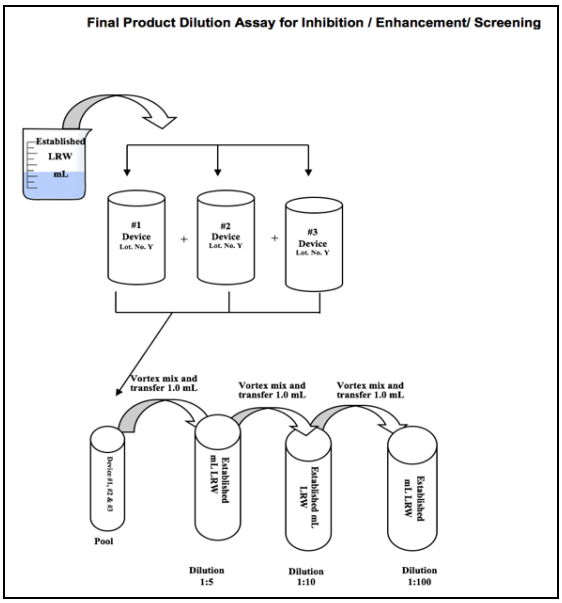

Figure 6
Final Product Dilution Series for Inhibition, Screening and Enhancement

Analyze

Evaluate times, quantity of vials used by CSE and reagents with Supplier 1 vs Supplier 2. An explicative diagram for both Supplier is shown below in Figure 7 and Figure 8.

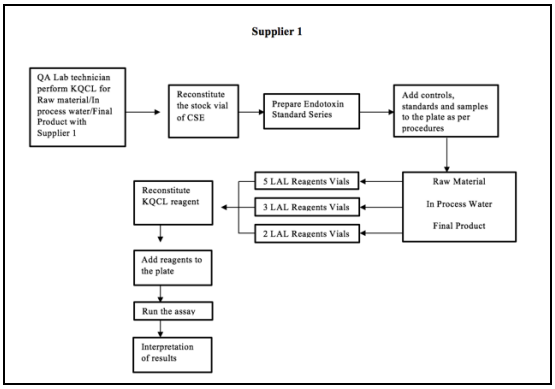

Figure 7
Actual State of the Process with Supplier 1

Currently, QA Lab conducts KQCL for raw materials, in process water, and final product according to our procedures. This test is performed using Supplier 1 reagents.

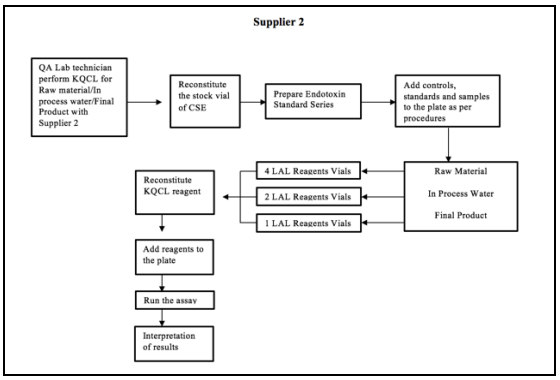

Figure 8
Supplier 2 State of the Process

Bacterial Endotoxin test by KQCL for raw materials, in process water, and final product will be conducted to validate Supplier 2.

Improve

Qualification and validation activities with reagents of Supplier 2 will be conducted as per regulations for raw material, in process water and final product. Also inhibition, enhancement and screenings will be performed for the same three (3) groups.

Control

Improvements on process will be completed and supplier 2 will be validated as per regulation to align the procedures with them. Also we must see a cost reduction.

RESULTS AND DISCUSSION

As established on methodology; using the six sigma process DMAIC, a Supplier 2 validation process were performed and the results is shown below.

Define

The supplier 1 service inefficiency, their quality issues was solved by the validation of Supplier 2 and their approval on procedures when the release process was completed.

Measure

Since the purpose of the study is to document strategy of Supplier 2 as an alternate reagent for KQCL; assays for raw material, in process water and final product where performed as explained on methodology and following our procedures. All of the samples used for raw material, in process water, and final product comply with specification criteria calculated as per USP <161>. The linearity of the standard curve demonstrated the Correlation Coefficient: between 0.980 to 1.000 and met USP regulation for initial standard qualification. Satisfactory results were obtained for the Standard Endotoxin concentration using of Supplier 2 Control Standard Endotoxin (CSE). Endotoxin recovered from the positive product control (PPC) is between 50% and 200%.

Analyze

When analyzing the process from start, yield reduction on time is 12 minutes shown on table 4 below.

Table 4
Time Analysis for the Reconstitution of CSE per Supplier

Standard Endotoxin	
Supplier 1	**Supplier 2**
E. coli O55:B5	*E. coli* O113:H10
Vortex CSE solution for at least 15 minutes at a high speed.	Vortex CSE solution for at least 5-10 minutes at a high speed.
The reconstitution volume will yield a CSE concentration of 50 EU/mL	The reconstitution volume will yield a CSE concentration of 1,000 EU/mL.
Dilute CSE stock to the appropriate standard concentrations, vortexing each glass tube for 60 seconds prior to making the next dilution.	Dilute CSE stock to the appropriate standard concentrations, vortexing each glass tube for 30 seconds prior to making the next dilution.

A similar analysis was conducted with Limulus Amebocyte Reagent; yield conduction is now reduced by 16 hours. Analysis is shown on table 5 below.

Table 5 bring an explanation of the specific time by process. That began by the lysate reconstitution with LWR and then the total time that the solution is stable.

Table 5
Analysis for the Reconstitution Volume and Stability Time of LAL Reagent per Supplier

Limulus Amebocyte Lysate reagent	
Supplier 1	**Supplier 2**
Reconstitution volume before use with 2.6 ml of LAL Reagent Water (LRW) per vial.	Reconstitution volume before use with 3.2 ml of LAL Reagent Water (LRW) per vial.
Reconstituted reagent is stable for 8 hours at 2-8℃ or can be stored at -10℃ for up to two weeks.	This solution is stable 24 hours at 2 - 8℃ or for two weeks at -20 ℃.

Improve

The qualification/ validation activities for KQCL assay with Supplier 2 were performed according to procedure ANSI/AAMI ST72:2011, USP <85>, and our procedures and the results were found satisfactory. Refer to Table 6 below for results.

Table 6
Results of Inhibition/Enhancement/Screening for Raw Material, in Process Water, and Final Product using KQCL Method with Supplier 2 Reagents

Product Description	Item No	Lot No	Maximum Validation Dilution	Product Dilution	Positive Product Control (PPC) Recovery	Acceptance criteria	Meet Acceptance criteria YES (Y) or NO (N)
Raw material Sample	1X	X	Calculated per ISO	1:10	All values results within acceptance criteria per regulations and our procedures.	50%-200%	Y
				1:100			
				1:1,000			
				1:10,000			Y
In Process Water	1X	X	Calculated per ISO	1:10		50%-200%	
				1:100			
				1:1,000			
Final Product	1Y	Y	Calculated per ISO	1:10		50%-200%	Y

Also an economic evaluation was performed and we have a favorable variance of $52,842.57 in a total of 183 lots that was tested. An explicative cost saving is shown below on table 7.

Table 7
Costs and Saving per Supplier

KQCL TEST					
Material used per One Lot					
Test Description	Number of vials Usage of Supplier 1	Supplier 1 Cost ($)	Number of vials Usage of Supplier 2	Supplier 2 Cost ($)	
Raw material	8	355.35	4	280	
In Process water	3	133.26	2	140	
Final Product	3	133.26	1	70	
Standard Control Series	7	310.93	3	156	Variable Favorable ($)
	Total	932.79	10	646	286.7864

Note: A calculation to conclude a favorable variance of $52, 482.57 for 183 manufacturing lots is shown below.

$$Cost\ Saving = (183\ lots)(\$286.79) = \$52,482.57$$

Control

Procedures were updated to reflect a new supplier Acceptance Criteria and implementation of usage of Bacterial endotoxin test/KQCL method using Supplier 2. The results for our combined manufacturing product with the Supplier 2 is a reduction on cost and the reduction on scrap materials.

CONCLUSION

The qualification/validation of this new supplier was made considering the necessities of the company and the manufacturing line requirements to comply with customer needed. This project was designed initially to have an alternate supplier instead on depends of only one. Then, an economic evaluation was performed and a yield reduce on the test time and cost was reflected. The yield reduces on test time help manufacturing line on received the product release faster and continue with their manufacturing steps. With this new validated supplier, a future projects could be conducted. For the same manufacturing product, we realize a pH assay and we have only one validated extraction solution. In the future we could validate a new supplier with another extraction solution for the final product.

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