Implementation of a Rapid Endotoxin Testing Platform

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Introduction

Bacterial endotoxin (or lipopolysaccharides), are structural parts of many Gram negative bacteria cell walls released during cell lysis that can create a pyrogenic (fever inducing) response in humans. All major compendia prescribe bacterial endotoxin testing (BET) and/or pyrogen testing to ensure that pharmaceutical products will be safe for use. The most common testing method is measuring the enzymatic reaction between bacterial endotoxin and the white blood cells of the horseshoe crab, *Limulus* Ambeocyte Lysate (LAL). By utilizing the interaction between LAL and bacterial endotoxin, a relatively quick (~15- to 60-minute) test can be used to determine the presence and level of endotoxin within a sample. There are currently three common methods using LAL:

- Gel-Clot (end point)
- Turbidometric (end point or kinetic)
- Chromogenic (end point or kinetic)

Bacterial endotoxin testing has evolved over the past 70 years, from measuring temperature change in laboratory rabbits based on pyrogenic reactions, to the monitoring of enzymatic reactions causing turbidity or color changes. As endotoxin testing platforms have improved, the opportunities for increasing assay speed, sensitivity, and/or decreasing the cost of testing, have continued to create more effective and efficient endotoxin testing systems.

An alternative rapid chromogenic endotoxin method has been implemented at both R&D and commercial manufacturing sites based on more than 3 years of cooperative work between multiple sites. This rapid method may provide a benefit over the current methodology and aligns with initiatives such as continuous improvement and zero defects. This paper provides a summary of the system and methodology, evaluation of data, the improvements/benefits achieved, quality systems in place surrounding the system, and examples of use.

Background

The motivation for switching to any alternative testing method is not only for comparability of the new method to traditional test methods, it is also important to consider a number of additional factors including: overall implementation costs, ease of use, time to run the method, and the ability to implement the method/platform globally. The overarching goal is to implement methods that comply with regulatory requirements while reducing sample handling, testing time, and deviations. Implementation of rapid test methods upstream in the process give added assurance of product quality and enable timely processing decisions. The rapid endotoxin methodology described here met all the criteria for accelerated results, robust testing, and overall enhanced product quality.

System Description

The endotoxin testing platform is a rapid and sensitive self-contained cartridge-based kinetic chromogenic endotoxin testing system. This platform measures absorbance using a spectrophometer and compares the observed value to a standard that has been calibrated and stored in the test system.

Novel to this system is the use of LAL-impregnated cartridges that allow for rapid analysis without the use of standard curves for each session. The platform also removes reagent preparation and handling (the reagents are self-contained in the cartridges), as well as exact measuring of reagents typical of traditional LAL assays.

Evaluation

In addition to typical assay evaluation exercises, several areas were assessed in regards to system robustness and operator impact of the assay. These included the variability/control of the positive product control (PPC) and impact of incorrect sample volumes by analysts.

Variability of Positive Product Control (PPC)

PPC, or sample spiking, is a requirement of the assay and provides feedback on assay performance and operator impact. Since the spiked endotoxin is a part of the impregnated cartridge, this is controlled not by the analyst, but by the system. Review of the PPC testing over three years of routine operation for ~47,000 samples has demonstrated the assay performs as designed and results are distributed across the compendial acceptance of 50% to 200% recovery. The study demonstrated statistically uniform distribution.

Figure 1 illustrates the PPC from 19 different colleagues' testing of several grades of pharmaceutical waters and in-process buffers. The average recovery was 100.5% with a standard deviation of 21%.

The graph of the PPC for a single operator, Figure 2, illustrates the stability of the assay over three years and the robust nature of the assay. The operator performed ~1800 assays and had an average recovery of 99.6% with a standard deviation of 21%.

Figure 3 illustrates the PPC of 222 samples from a single Water for Injection (WFI) sample point over three years of testing (multiple analysts). The average recovery from this study was 99.2% with a standard deviation of 17%.

Impact of Sample Volume

During the initial assessment of the system, consistent and accurate sample addition was a concern for assay performance. As the reagents are already impregnated into the cartridge, the analyst only controls the sample volume, which can impact the final concentrations of the reagents/endotoxin in the test. Over or under diluting the reagent may produce inaccurate results. Studies were conducted which demonstrate deviating from the required sample volume would be evident and reported by the system due to invalidation of the test by not meeting the required 50-200% recovery. The sample volume study evaluated sample volumes ranging from 10 to 35 µl and found that a sample volume of 25 µl consistently met the PPC acceptance limits. Volumes greater than or less than 25 µl resulted in invalid assays (see Figure 4). Insufficient sample volume results in overly concentrated spiked samples (>200% PPC recovery) and in the same fashion, too much sample overly dilutes the PPC (<50% PPC recovery).

Implementation

The rapid kinetic chromogenic endotoxin assay is compliant with USP <85> and EP 2.6.14; therefore use of the assay requires only analyst training and verification of product performance. After the completion of site specific qualification of the equipment and operator training; additional product specific experiments for potential assay enhancement/inhibition interactions are performed. Evaluation of the PPC recovery and coefficient of variations (CV) are performed to establish method/product compatibility. The following criteria must be met to be considered acceptable:

- PPC percentage of the positive control between 50% and 200%
- CV of Sample <25%
- CV of PPC <25%

Figure 1 - Positive Product Control (PPC) Composite Sample Results from Water and Buffer Testing

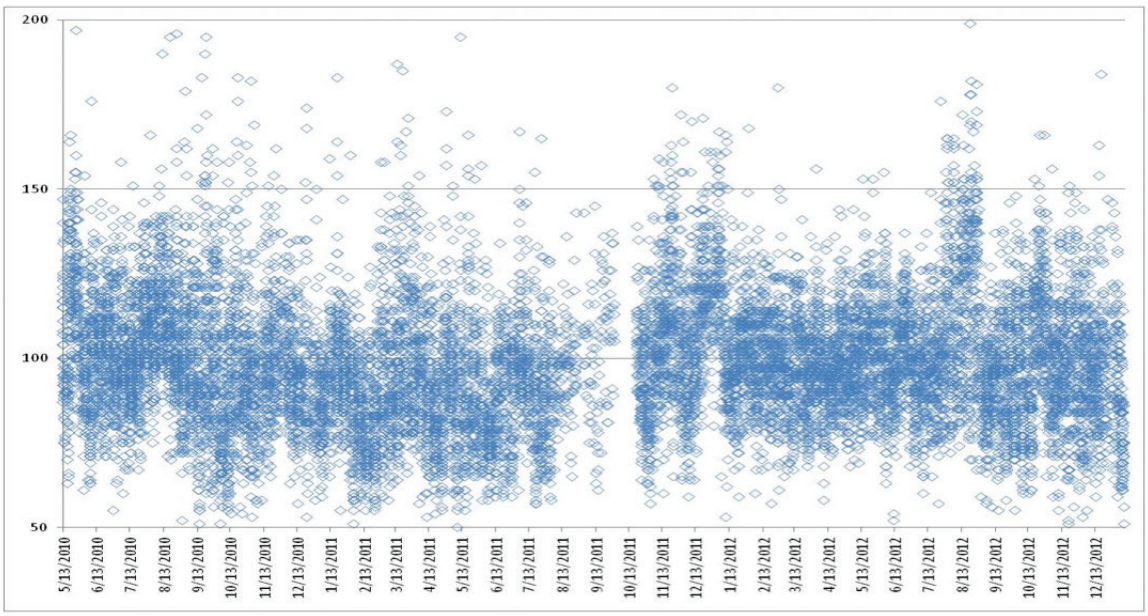


Figure 2 - Positive Product Control (PPC) for a Single Operator

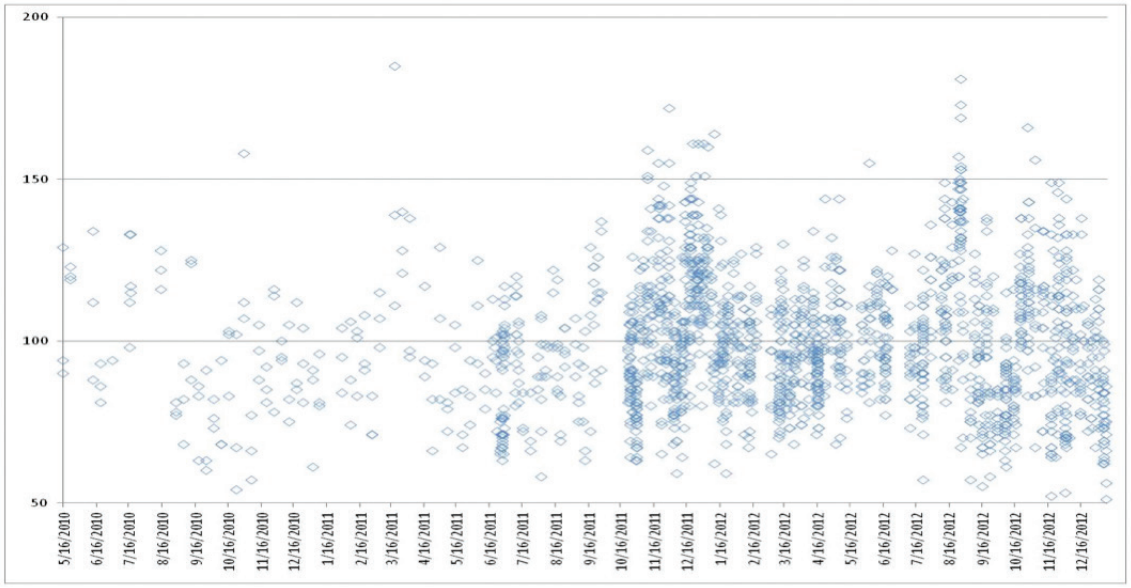
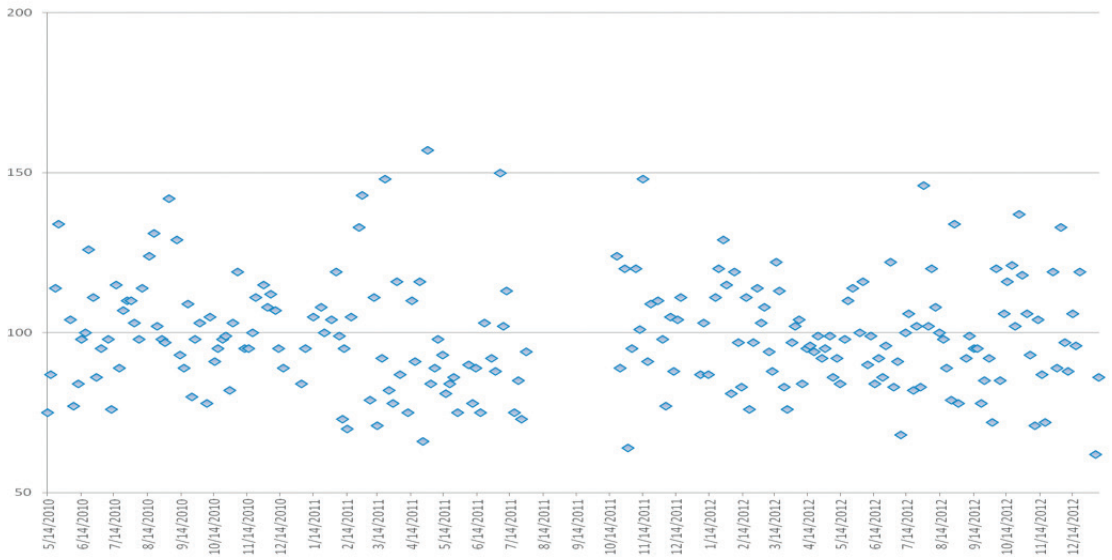


Figure 3 - Positive Product Controls (PPC) for a Single WFI Sample Point over 3 Years

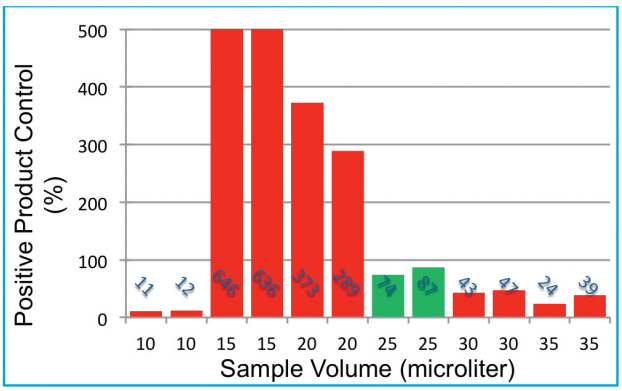


Per compendial requirements, assay verification for routine use requires one lot of material to be assayed within assay parameters (passing of CV and PPC criterion); however, three lots of material are frequently evaluated to demonstrate consistent assay performance.

Additional points to consider when implementing the cartridge-based endotoxin testing with specific products include pH, negative controls, and review of PPCs.

Due to the nature of the impregnated reagents and small volumes used, verification of the pH for appropriate use of the assay is challenging. Per USP <85>, the sample pH should be adjusted so that the pH of the mixture of the lysate and sample solution falls within the pH range specified by the lysate manufacture, usually 6.0 to 8.0. According to the rapid endotoxin cartridge manufacturer instructions, the pH of the test specimen *prior to testing* should be measured to assure a pH within the

Figure 4 - Sample Volume Study Results



range of 6.7 to 7.3. Therefore, if the sample pH is within the tightened pH range (6.7 to 7.3) for the rapid test system, it is not necessary to perform additional pH measurements or adjustments of the sample in the presence of LAL reagent as described in USP <85>.

Negative controls are a fundamental requirement for endotoxin testing which demonstrate there is no extraneous source of endotoxin introduced by the assay. Traditional test methods normally require negative control testing be performed for each testing session. However, the discrete nature of the platform indicates that negative control testing of the water for the sample dilution is sufficient and should be tested once per lot of endotoxin test cartridges and once per lot of LAL Reagent Water (LRW).

PPC testing demonstrates the assay performs as designed when results are distributed across the acceptance of 50% to 200% recovery. Periodic review of the PPC results for each product tested is recommended to ensure the results are not biased toward the extremes of the recovery range. While different dilutions of the sample solutions should not impact the PPC of the product, it is advised that during implementation of the assay, different dilutions be evaluated to demonstrate robustness of the PPC measurement.

System Applications

While the system has shown comparability to the classic LAL chromogenic assay, the implementation of any alternative and rapid microbiological method systems requires reliable assays and the development of effective quality systems oversight to ensure consistent and robust use of methods. The alternative rapid method was implemented globally in accordance with corporate quality policies and site Standard Operating Procedures.

Testing units were purchased and qualified leveraging vendor supplied qualification protocols. Additional qualification and method validation requirements were performed, as needed, for specific applications and specific products. Laboratory technicians were trained and qualified on the new equipment and methods, similar to traditional method training. Consumable materials (ie, test cartridges) are controlled within the quality system with purchasing specifications and release testing performed to ensure the quality of the test material. In addition, cartridges are dispensed through the quality system thus preventing unauthorized repeat testing should an unexpected result be obtained. The systems are maintained under GMP change control to ensure upgrades or changes are appropriately assessed prior to implementation.

Business Benefits of the System

The rapid method afforded numerous benefits to the business, including a reduction in assay costs, at-line/near-line monitoring to reduce handling, and a measurable decline in the number of deviations and investigations associated with endotoxin testing. Due to reduced manipulations and the automated nature of the system, the amount of operator training and the potential for unintentional operator bias of BET testing assays is significantly reduced. In addition to the direct business benefits, the rapid testing platform has the potential to enhance product quality by utilizing the rapid test upstream of final product thereby identifying and remediating areas of concern well before the product is ready for release.

Typical laboratory technician time for classic endotoxin testing for set-up, calibration, testing and clean-up is approximately 4 hours—regardless of the number of test samples managed by the manufacturing site. This applies to gel-clot, turbidometric, or chromogenic testing. Assay time for the cartridge-based systems, however, is approximately 15 minutes, representing a large reduction in the time to result. Across the manufacturing network, the assay cost savings was most evident for those sites where testing volumes were lower—for example, sites that performed <5 endotoxin tests per day. For these lower volume sites, there was a measurable savings in test method reagent costs, as they were able to reduce their stock levels and discard rates for short shelf life reagents.

Surprisingly, the high volume test sites (>30,000 tests per year) demonstrated the most savings due to a decrease in laboratory investigations and operator variability/training. In general, the simplicity of the rapid endotoxin test has resulted in a reduction in the number of laboratory deviations and investigations. One manufacturing site reduced their laboratory deviation rate by more than 90% when they implemented the rapid test. At an estimated savings of approximately $2000 per investigation, the savings have been significant.

The rapid cartridge based system may be ideal for at-line or near-line testing, especially where potent materials are handled. The risk of personnel exposure and facility contamination is minimized by keeping the sample preparation and testing in close proximity to the operation. In any application, the use of at-line/near-line testing eliminates the time to transport the sample to the laboratory and coordinating the testing within the lab.

Conclusions

The rapid endotoxin testing platform was successfully implemented across multiple sites and is used for a variety of purposes including water, raw material, buffers, cleaning verification, in-process, and final product testing. The technology was centrally assessed for suitability and comparability to compendial methods. Data from ~47,000 samples taken over a three year period, representing a variety of samples and numerous laboratory technicians was evaluated to support the reproducibility and robustness of the test system. Additionally, work was done to understand the importance of sample volume. Systematic assessment, evaluation, qualification and development of processes have allowed global implementation of the rapid endotoxin test system in accordance with quality systems.