Bioscience Solutions

Streamlining Your QC Testing: The Efficiency and Accuracy of Automated Endotoxin Testing and Process Optimization

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Abstract

Endotoxin Testing is an essential quality control (QC) test used across the medical and bioscience industries to ensure safety of medical products used to treat human and animal patients. Errors or inefficiency in these testing steps can create unacceptable backlogs in production and significantly affect both total costs and timely product release. Multiple test methods exist for endotoxin testing, and regardless of which method a lab is using, the tester will be required to perform numerous pipetting and data entry steps. Labs which have not automated these pipetting and data entry steps require additional resources to perform steps that are tedious and error prone.

To address these issues, Lonza has created a next-generation Automated Endotoxin Testing platform that through process optimization and automation of routine manual tasks associated with endotoxin testing will be able to streamline and improve the performance of the QC laboratory. The automation points are targeted to maximize return on investment through savings in time and the reduction of error associated with performance of manual tasks, and overall cost savings.

The Automated Endotoxin Testing platform is a modular platform product based on our WinKQCL™ Endotoxin Detection software. This is a comprehensive custom solution comprising robotic liquid handling systems, endotoxin readers and reagents, and interconnected software applications to robotics management software, LIMS, CAPA systems, or our MODA™ EM Task Manager, and emphasizes a flexible, configurable means of sharing data and tasks between systems.

In this poster, data will be shown demonstrating the ability of robotic systems to reproducibly and reliably perform the preparation of standards, complex routines and dilution schemes necessary to endotoxin testing, and to demonstrate the efficiency, accuracy, and effectiveness of an automated solution when implemented in a large-scale fashion.

Introduction

Endotoxin Testing is an essential quality control (QC) test used for release testing in the pharmaceutical industry to ensure the safety of parenteral drugs and medical devices, as well as a commonly executed quality control test for countless other raw material products sold into these markets. However, because of the strict precision required and yet the routine nature of the testing process, human error creeps into this process regardless of the best efforts of managers and staff. Nonetheless, the routine nature of endotoxin testing also makes it amenable to an automated solution, which can reduce the potential for human error substantially. A full implementation of an automated solution for endotoxin testing will likely lead to the streamlining and improvement of performance in the QC lab, and through process optimization, result in time savings, fewer errors, and overall cost savings.

In light of the increased demand for more thorough and comprehensive endotoxin testing, especially for large-volume manufacturers, Lonza has created an add-on automation module to its industry-leading WinKQCL™ Endotoxin Detection software. The WinKQCL™ Automation Module simplifies the process of setting up an automated endotoxin testing run and supports selection and use of existing WinKQCL™ Templates. When a WinKQCL™ Template or templates are selected, a layout map of the robot deck is displayed. The map provides instructions for positioning labware and reagents on the robot deck and also calculates and displays the required volumes for each liquid used in the assay. Block components displayed on the deck layout map correspond to physical grid locations on the robot deck. The map includes a software checklist with each item in the checklist corresponding to an item on the robot deck. Each item in the checklist must be confirmed (location, volume, etc.) prior to running the assay.

Upon initiation of the assay, the robotic control code to perform the assay is generated dynamically, based entirely on the selected WinKQCL™ Template (no programming or robotic scripting knowledge is required). Once the automated assay is initiated the analyst can walk away. The robot executes all processing required to complete the run, including microplate transfers in and out of the reader at the appropriate times for incubation. Once the microplate setup is completed, the microplate is transferred to the reader, and when the assay is finished, the WinKQCL™ Software automatically reads the microplate and saves the results to the database. The WinKQCL™ Automation Module provides a simple and reliable front end to the robotic process, and removes or greatly reduces the potential for human error in the setup, pipetting steps, and calculations.

In this poster, we show the ease and utility of the software in setting up standard curves and complicated dilution schemes, and the accuracy of the robotic system in pipetting standards and samples. In addition, we show data from one of our customers who has implemented an automation solution, albeit in a semi-automated fashion. However, the data readily demonstrates the feasibility and accuracy of robotic pipetting in a large-scale setting, and can demonstrate the advantages of an automated solution of the savings in time and the reduction of error associated with performance of manual tasks, and overall cost savings.

Materials

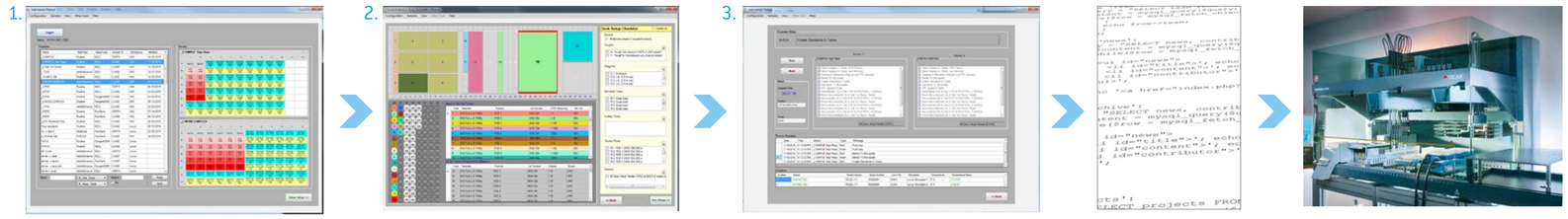
Equipment: The hardware used in this study consisted of the Freedom EVO®150 robotic platform, utilizing the Freedom EVOware® Software, Version 2.6, Service Pack 3, the Tecan Sunrise™ Plate Reader, the Lonza WinKQCL™ Endotoxin Detection and Analysis Software, Version 5.5, along with the Lonza Endotoxin Automation Software Module, Version Alpha.

Reagents: Lonza Limulus Amebocyte Lysate Kinetic-QCL™, Cat. No. 50-650U; Lonza USP Reference Standard Endotoxin (RSE), Cat. No. E700; and Lonza LAL Reagent Water, W50-100 or equivalent.

Consumables: Lonza Pyrogen-Free Dilution Tubes 13 x 100 mm without Caps, N207; Lonza LAL Reagent Grade Multi-well Plates, Cat. No. 25-340; Lonza Eppendorf Biopur® Pipette Tips, 1000 µL, Cat. No. 25-417; Lonza Eppendorf Biopur® Pipette Tips, 200 µL, Cat. No. 25-415; and Lonza Endotoxin-free Reagent Reservoirs, Cat. No. 190035.

Standard Preparation: Standard curves were generated by resuspending the CSE from the LAL Kinetic-QCL™ Kit to 50 EU/mL in LAL Reagent Water, and by directing the software and robot to create a serial dilution 5-point standard curve of 50 EU/mL, 5 EU/mL, 0.5 EU/mL, 0.05 EU/mL, and 0.005 EU/mL. Manual curves were generated by similar serial dilutions of the 50 EU/mL standard to generate the 5-point standard curve.

Reaction Time Summary Statistics Over 12 Assays
Figure 1

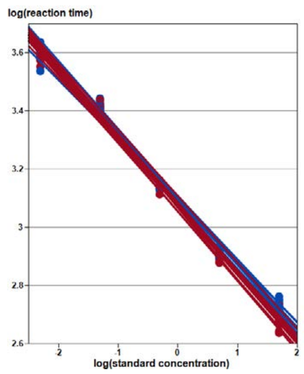


1. Select the WinKQCL™ microplate template(s) to be used in the run
2. Setup and confirm the robot deck layout
3. Initiate and monitor the assay
4. Robotic control code dynamically generated based on template(s)

Reaction Time Summary Statistics Over 12 Assays
Figure 2A

Standard EU/mL	Mean Reaction Time		Standard Deviation Over Assays		Pooled %CV Among Replicate Wells	
	Manual	Auto	Manual	Auto	Manual	Auto
50	481	536	±28	±29	2.9%	2.8%
5	794	815	±29	±24	2.2%	1.2%
0.5	1 371	1,377	±38	±28	1.8%	1.1%
0.05	2 60	2,576	±77	±79	1.1%	2.2%
0.005	3 904	3,991	±166	±213	2.4%	1.4%

Standard Curves Generated over 12 Assays: Manual and Automated
Figure 2B



Customer Data Demonstrating a Large-scale Implementation of a Robotic Pipetting Solution
Figure 4

	Sample Size	Passing	Average	% Passing
Standard Curve Correlation	25,819	25,734	-0.999	99.67%
Standard Curve Slope	25,819	25,734	-0.241	99.67%
Average Standard %CV	129,095		1.07%	
Assay Pass/Fail	542,199	527,954		97.40%
Average Sample %CV	204,394		1.19%	

Sample Preparation: Samples were generated by resuspending the CSE from the LAL Kinetic-QCL™ Kit to 50 EU/mL in LAL Reagent Water, and diluting down to 1 EU/mL. These samples were used to create a dilution series of 1:10, 1:100, 1:1000, and 1:52. Corresponding samples also included a 0.5 EU/mL PPC spike. These experiments were repeated 5 times, and the results pooled and analyzed.

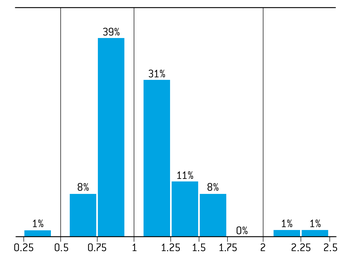
Results

In Figure 1 we show a flowchart of the steps required to execute an automated endotoxin testing run. (1.) Select the WinKQCL™ Microplate template(s) to be used in the automated run. The list of microplate templates is imported directly from the WinKQCL™ Database. The worklist templates are imported to WinKQCL™ Software from a central database such as LIMS, CAPA system, or our MODA™ EM Task Manager. Dilution and PPC requirements specified within each template will be accommodated by the software. (2.) Setup and confirm the robot deck layout. (3.) Initiate the assay. Results may be exported to a LIMS, CAPA, MODA, or other system if desired, emphasizing the flexible, configurable means of sharing data and tasks between systems.

Figure 2 shows a comparison of 12 standard curves generated either manually or by the robot. In Figure 2A, we see the mean reaction times for the 12 manual and robot-generated standard curves. As seen in the % CV's for the standards, all of the CV's are below 3%, and most are near 2% CV or below, well within industry standards. Figure 2B shows all 24 manual and robot-generated standard curves superimposed, with robotic standard curves in blue and the manual standard curves superimposed over these in red. All of the standard curves pass industry standards and are valid curves. These data indicate that the robot-generated standard curves and the manually generated standard curves are comparable.

Figure 3 shows the results from a series of fully-automated runs. A 1 EU/mL sample was diluted and tested for recovery, and in each case, complemented by the corresponding sample containing a 0.5 EU/mL spike. In Figure 3A, each 1 EU/mL sample was tested either undiluted, or diluted 1:10, 1:100, 1:1000, or 1:52, and the EU measurements calculated. Of the 85 measurements, 97% fell within the 50%-200% recovery range (black lines). In Figure 3B, the same samples and dilutions also contained a 0.5 EU/mL PPC spike, where 100% of the 103 measurements fell within the 50%-200% recovery range (black lines).

Assay Measurements on 210, 1 EU/mL Samples Over 5 Assays
Figure 3A



PPC Recovery Measurements on the Samples Containing a 0.5 EU/mL PPC Spike
Figure 3B

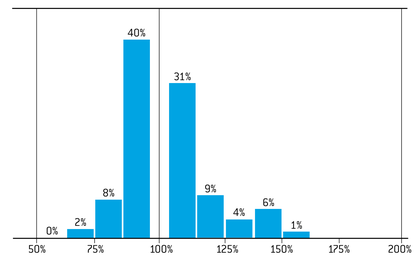


Figure 4 shows data obtained from a customer who has implemented a large-scale automation solution. Although this customer has opted for a semi-automated robotic solution, this system has been in place for over a decade, and is an excellent example of the accuracy and consistency available with a robotic solution. Of the over 25,000 standard curves that were set up in 2015 at this customer site, 99.67% of the standard curves passed, with an average %CV of 1.07%, demonstrating effectively the results obtainable with robotic pipetting. They tested over 500,000 samples during 2015 (samples and samples + PPC), with a passing rate of over 97% and a 1.19% CV (for samples alone).

Conclusions

Endotoxin testing plays a critical role in the release testing of parenteral drugs and medical equipment, as well as in the testing of raw materials entering the manufacturing process in the pharmaceutical industry, and bottlenecks at this point of manufacture can become rate-limiting steps in the efficient manufacture and release of these products.

Because of the increasing demand for monitoring and testing, Lonza has created the Lonza Endotoxin Automation Software Module as an add-on to its industry-leading WinKQCL™ Software in order to facilitate the development of an automated system requiring minimal human intervertion for performing endotoxin assays that also provides performance that is equivalent to, or better than, the performance of assays that are manually executed by an experienced technician.

In this study, we demonstrate the ability of the Lonza Endotoxin Automation Software Module to accurately generate standard curves and easily handle complex dilution schemes. The simplicity of the WinKQCL™ Automation Module software requires no programming or robotic scripting knowledge, and results can be easily transferred into and out of existing LIMS, CAPA, MODA, or other databases.

The simplicity of the setup of the robotic deck with the Endotoxin Automation Module is balanced with the demonstrable accuracy of robotic pipetting in a large-scale format. As seen with the data from our customer, the consistency and accuracy of both standard curves and samples in a robotic environment is formidable in both quality and quantity.

Therefore, the WinKQCL™ Endotoxin Automation Software Module coupled with the appropriate robotic system lays the foundation for a fully-automated endotoxin testing platform, with complete interconnectivity with a variety of client databases, that should lead to an increase in throughput and accuracy, reduction in human error, reduction of the working time required by technicians to implement testing, reduction of ergonomic stress and repetitive strain injuries, and an overall improvement in efficiency.

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