Automating the BET with Dynamic Scripting
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Pharma & Biotech

Automating the BET with Dynamic Scripting

Lonza patented and innovative dynamic scripting with the PyroTec® PRO Automated Robotic Solution provides a flexible, robust and user-friendly automation system for BET assays.

PyroTec® PRO System simplifies automation of the BET assay

Introduction

Bacterial Endotoxin Testing (BET) is an essential Quality Control (QC) test for raw materials, in process, and final release of all injectable drugs and implantable medical devices. The majority of BET testing is conducted manually which, given the multiple laborious and repetitive pipetting steps, is inherently error prone and has a high training requirement. Manual BET testing carries a high repetitive strain injury risk especially for high-throughput laboratories such as contract testing labs and large manufacturing sites. There are significant data integrity challenges when manual processes are employed including the lack of metadata associated with the manual steps and manual transcription of data into systems such as a Laboratory Information Management System (LIMS) or the Lonza MODA® Solution.

Early automation – the PyroTec® Robotic Solution

Some companies, including Lonza, began considering automation of BET over a decade ago but this was limited in scope due to the special difficulties associated with routine samples. Existing generic automated liquid handling systems are designed for high-throughput testing of similar test articles with the same test, while endotoxin testing requires testing of complex sample matrices and different treatments. Samples for BET typically have widely varying dilution factors, sometimes need additives (e.g. $MgCl_2$) and may have diverse liquid characteristics (e.g. viscosity). Early attempts to automate BET such as Lonza's PyroTec® Robotic Solution required extensive programming skills to generate separate static scripts in the application for the robot to be able to prepare different templates (i.e. the same components must be positioned at the same locations each time the template is executed, meaning processing is always the same). Frequent manual script writing is a potentially error prone process which is both labor intensive and requires a high level of training. The time taken to create each script impacted the agility to respond to changes in workflow or sample type. As a result, early robotic BET solutions were limited primarily to simple testing of water and dialysis samples.

Automating BET: the next generation

In 2016 Lonza's development team began to design and develop a next generation BET automated solution that could handle the complexities of BET assays. The team discussed customer needs with the Field Application Service team and determined that the ideal BET automated solution should simplify the process to such a degree that no end-user programming expertise would be required. In other words, BET automation should be as simple as selecting the plate template, loading the deck with the required labware, samples and reagents, initiating the automated run, and walking away (Figure 1).

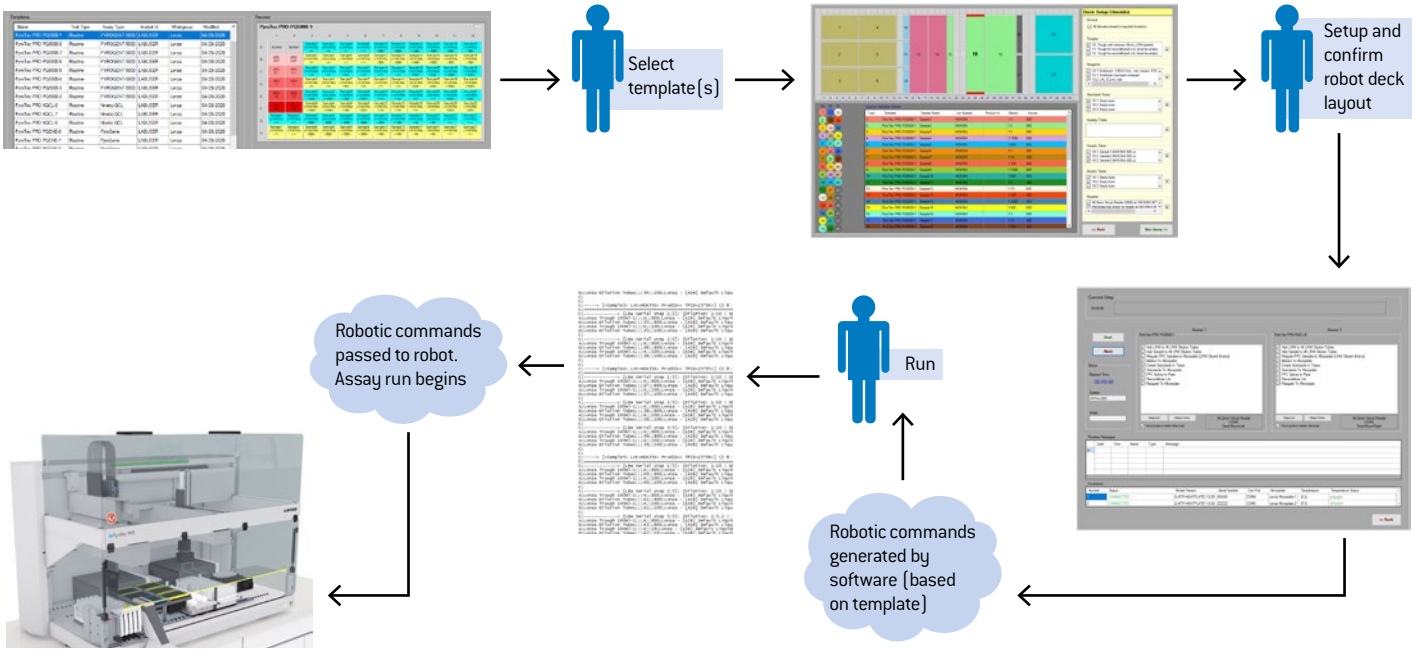

Figure 1: The 3-step "walkaway" automated assay process flow.

Introducing Dynamic Scripting

Traditional automated endotoxin testing systems are based on a static design approach, whereby pre-existing control code contains the instrument instructions required to perform an automated assay. The same assay must be performed using the same deck layout every time. The static, inflexible nature of pre-existing static control code makes the functionality of traditional systems limited and narrow in scope.

Due to the inflexibility of static design systems, users are faced with an ongoing challenge. When processing changes are required (e.g., to perform a different or modified assay, adding new sample types, etc.), they must be applied by a person who has specialized knowledge and experience with automation scripting and/or software coding. Process changes can be time-consuming and error-prone which is neither desirable nor acceptable in pharmaceutical or medical device quality control (QC) testing laboratories.

Lonza's design for automating endotoxin assays is premised on a novel dynamic control code approach that contrasts starkly with the traditional static design approach. In many ways, Lonza's design approach is, in fact, the inverse of the traditional static design approach. As opposed to pre-existing control code, Lonza software algorithms dynamically construct the required robotic control code (and deck layout information) based entirely on the assay template selected by the user. Many components of the Lonza design are configurable as demonstrated in Figure 2 below.

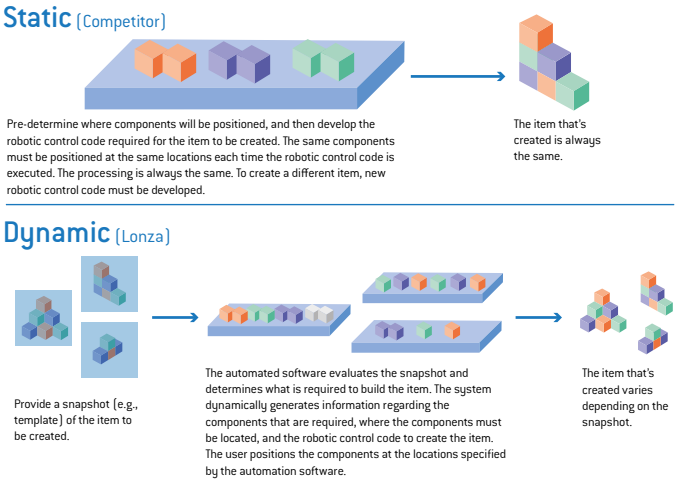

Figure 2: Static versus Dynamic Scripting

Lonza developed the PyroTec® PRO Automation Software Module to provide automated BET and dynamic scripting. The module is available as an 'add-on' to Lonza's existing WinKQCL® Software and is able to evaluate the template and determine what is required to perform the automated assay. Information about all of the required components (samples, diluents, reagents, labware, and consumables), where they should be located, and the robotic control code to perform the assay is dynamically generated by the required PyroTec® PRO Automation Software Module. The deck layout information specifies labware (and positions) required for the assay along with calculated required liquid volumes. Confirmation that the deck layout has been set up in accordance with the generated "map" (Figure 3) safeguards against commencement of an assay with incorrect or improperly positioned labware. The user simply populates the robotic deck according to the software-generated "map" and initiates the automated assay(s). The WinKQCL® Software template may be saved for re-use or the user may create a new unique template for each assay.

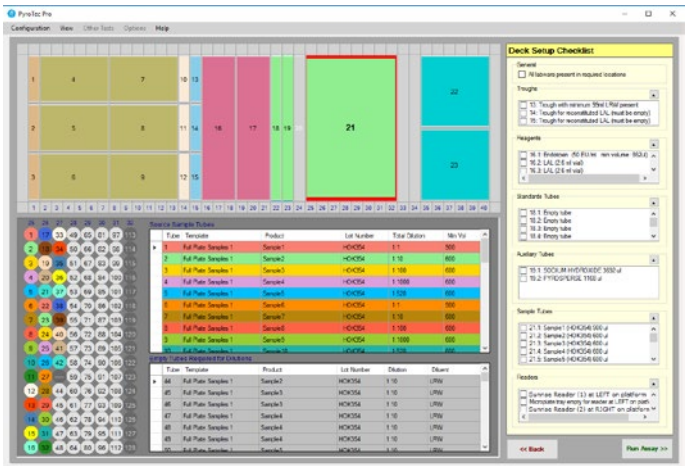

Figure 3: Robot Deck Layout

The PyroTec® PRO Automated Robotic System dynamically generates the robotic commands to fully automate all steps of the endotoxin assay:

1. Sample dilutions
2. Placing of samples on the plate
3. Placing water blanks on the plate
4. Creating dilutions for standard curves
5. Placing standards on the plate
6. Adding Positive Product Control (PPC) spikes to the correct wells
7. Moving the plate to the reader for pre-incubation
8. Preparing the reagents
9. Moving the plate from and to the reader to the on deck incubator
10. Adding reagents to wells
11. Moving the plate back to the reader for processing
12. Reading the plates and processing the results

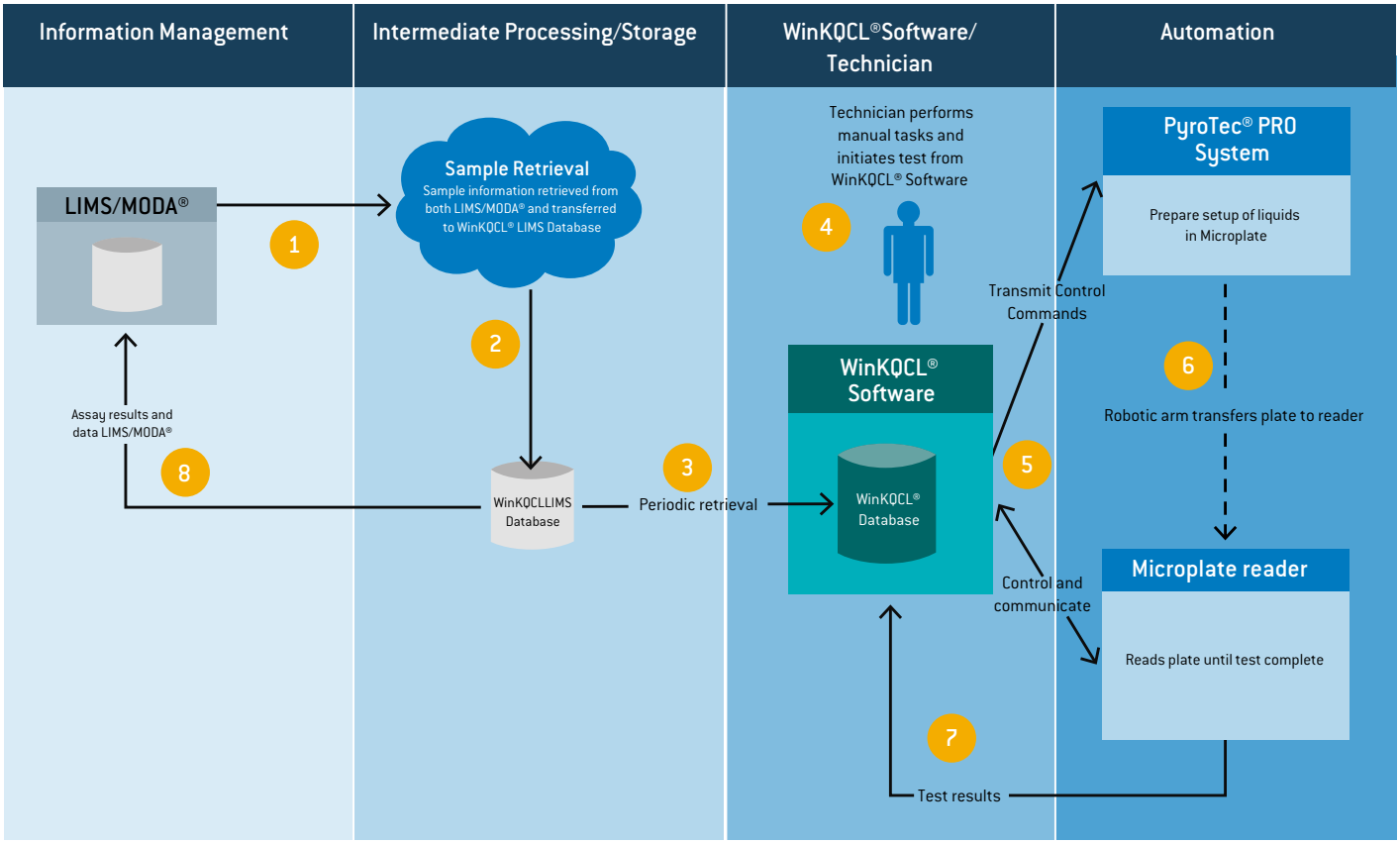

Figure 4: An example of incorporating BET automation into laboratory process flow

The future of Dynamic Scripting – The PyroTec® PRO Robotic Solution for endotoxin detection

Dynamic scripting allows minimal hands-on intervention by the lab analyst and does not need any scripting work to be carried out for routine testing, reducing the training load, and increasing the ability of the lab to respond to any changes in routine, samples, or acceptance criteria. The seamless integration of WinKQCL® Software and PyroTec® PRO System with application software, firmware and device drivers on the robotic deck means that the user only needs training and skill using WinKQCL® Software and PyroTec® PRO System.

1. Sample information is periodically retrieved from the MODA® System or LIMS by a scheduled task running on the WinKQCL® Software host computer.
2. The retrieved sample information is transferred to the WinKQCLLIMS database by the same scheduled task as specified in step 1.
3. Sample information stored in the WinKQCLLIMS database is periodically retrieved by a technician and imported into the WinKQCL® Software to begin setup for testing of the sample.
4. The technician manually places physical samples, additional required liquids, and required labware on the instrument deck, at the volumes and locations specified by the PyroTec® PRO System software module. The technician then initiates the start of the assay.
5. The PyroTec® PRO System software module generates and transmits the appropriate commands to the PyroTec® PRO Automated Robot to perform the assay.
6. Following completion of the microplate setup, the PyroTec® PRO Automated Robot transfers the microplate to the microplate reader (via robotic arm). The microplate reader reads the wells of the plate according to the assay type selected in the WinKQCL® Software template.
7. WinKQCL® Software receives optical density reads from the microplate reader at each time-point, assimilates the data and determines when the test is complete.
8. WinKQCL® Software transfers test data and other information to the MODA® System or LIMS. Using the WinKQCL® Software platform for all BET tasks means all data and metadata for each assay, performance of the system, etc. are easily found in the audit trails and log files (i.e. one place allowing for easy reporting and trending activities). Two-way integration of the WinKQCL® Software with lab information systems such as the MODA® System or LIMS further simplifies the labs workflow, reducing the potential error associated with manual data transcription. Configuration of the software to automatically upload sample worklists is possible to ensure samples do not get missed and enhance SOP compliance.

Table 1: Comparing Static Design and Lonza's Dynamic Scripting

Characteristic	Static Design	Lonza Design
Robotic Control Code	Static and predetermined (processing modifications require modification of the existing robotic control code by an individual with specialized knowledge and skills)	Dynamically created to accommodate the selected template (no modifications to robotic control code required regardless of assay)
Deck Layout	Assay labware must be positioned at pre-determined deck locations	Both the deck layout and assay labware locations are configurable
Liquid Sample Handling	The types of liquid samples to be tested are predetermined and limited	Any type of liquid sample that can be handled by the robotic hardware can be configured and tested on the system
Liquid Volumes	Predetermined	Calculated to accommodate the selected template and reported to the user
Sample dilutions and diluents	Predetermined and limited	All sample dilutions specified on the template are supported, diluents used are configurable. Dilutions requiring multiple diluents and processing of additives (such as PYROSPERSE® dispersing agent) are supported
Assay Flexibility	The same assay is always performed in the same way (possibly with minor variations)	The assay to be performed is variable, determined by the template selected by the user
Resulting Plate Layout	Always the same	Varies according to the selected template

Lonza's PyroTec® PRO Robotic Solution encompasses highly flexible sample configurations (48 available tubes for samples, 80 tubes available for dilutions per run), low assay setup time (<10 minutes/42 samples) whilst retaining compliance with the relevant BET Pharmacopoeia by maintaining standard curve integrity (Table 1). The ability of the system to automate primary (LAL reagent water (LRW)) and auxiliary dilutions (e.g., beta-glucan blocker) allow simple and viscous sample types to be run simultaneously. Configurable liquid classification allows the user to test extremely viscous sample types which can require advanced pipetting skills when done manually. Strong repeatability statistics showcase a high accuracy/low coefficient of variation between replicates (Found in our Whitepaper – "It's All Mixed Up" Allen L. Burgenson, John Cooley, Lonza Walkersville, Inc., and available for download in our QC Insider™ Toolbox.

WinKQCL® Software and the PyroTec® PRO Automated Robotic Solution unique dynamic scripting invention is now patented with the United States Patent office under Patent No US 10,768,190 B2, *Dynamically Controlling an Automated Assay*.

Conclusions

Lonza's patented and innovative dynamic scripting process has greatly simplified the automation of the BET assay, making it a more robust process. Automation of BET greatly reduces pipetting and plating errors, such as sample misplacement and volume mistakes, while freeing laboratory analysts from repetitive motion tasks and their inherent risk of injury. Less hands-on assay time allows the analyst to perform higher value tasks while the robot performs routine sample preparations and assay incubations. The Dynamic Scripting with the PyroTec® PRO Automated Robotic Solution provides a more flexible, robust and user-friendly automation system for BET assays.

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RT-SP026 03/21

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