Optimizing and Future-Proofing Bacterial Endotoxin Testing with Flexible Automation

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Abstract

Bacterial Endotoxin Testing (BET) is a fundamental requirement in the biomedical and pharmaceutical industries to ensure patient safety. However, both sample types and assays can be highly variable, making automation a challenge, so most laboratories still perform BET manually. As with other manual laboratory processes, this makes BET cumbersome, prone to human error, and difficult to integrate with laboratory information management systems (LIMS), leading to poor data integrity and sample traceability. The high number of pipetting steps also makes manual BET potentially harmful, presenting an increased risk of repetitive strain injury (RSI). As such, laboratories have urgently sought to automate the BET process.

The generic automated solutions that attempt to address manual BET challenges are typically limited to a single defined workflow, where any process modification requires tedious re-writing of automation scripts. While this kind of static automation is suitable for a small subset of end-users, most laboratories require flexible automation that can accommodate the full diversity of BET samples and assay types – without the need for frequent script re-writing and deep end-user programming knowledge.

Bacterial Endotoxin Testing: Still A Manual Task

As a structural component of the cell wall of gram-negative bacteria, endotoxins are ubiquitously present in the environment. Bacterial endotoxin contamination in pharmaceutical products can cause fever and septic shock in patients, and may even be fatal in the most severe cases. Testing all parenteral medicines, vaccine preparations, and injectable or implantable devices for the presence of endotoxins is a stipulation of Good Manufacturing Practices (GMP) and must comply with requirements enforced by the global pharmacopoeias. Most companies will also test raw materials and in-process samples for endotoxins as part of their quality control (QC) strategy to better ensure patient safety.

BET is usually performed manually, as sample matrices tend to be complex and often require varying dilution factors, diluents, or additives to overcome assay interferences and ensure reliable results. Some samples are also highly viscous and therefore challenging to

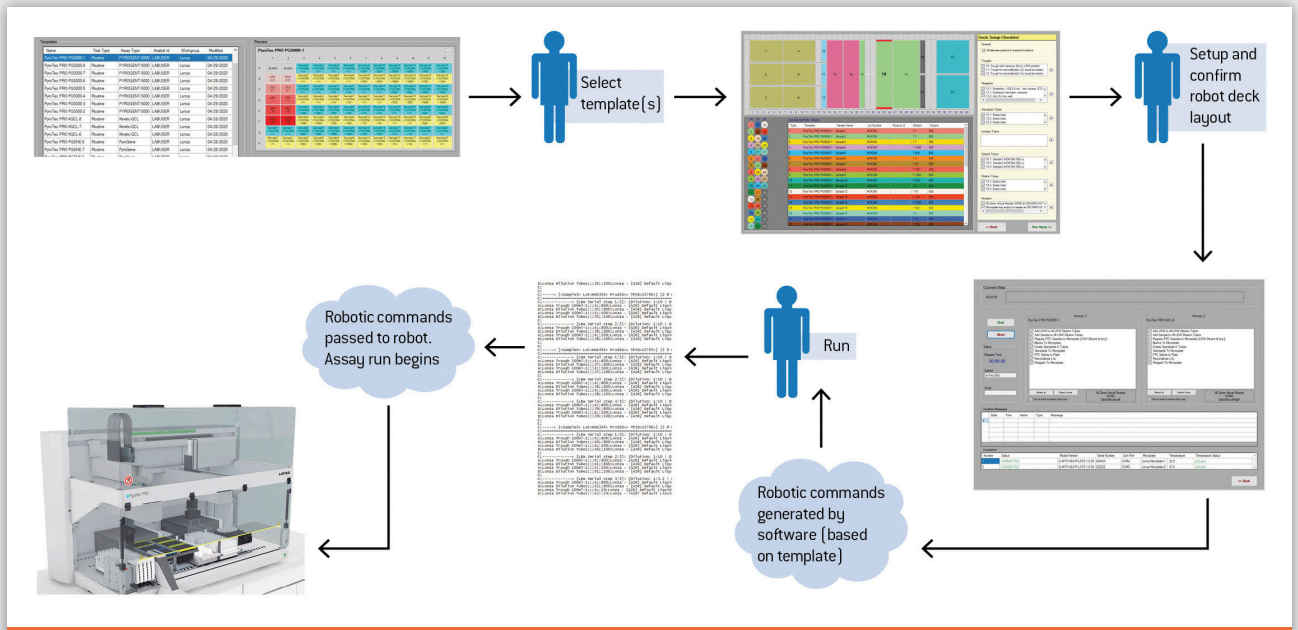

Figure 1. Example of a flexible automated assay process flow, requiring only three manual interaction steps.

pipette, even in a manual setup. Additionally, different sample matrices may work best with particular assay types – for example, a laboratory may choose from the traditional chromogenic or turbidimetric Limulus amebocyte lysate (LAL) assays or the more recently developed sustainable assays based on recombinant Factor C.

As with most laboratory applications, manual procedures have significant downsides. They are labor-intensive, preventing staff from taking care of more value-added work, and carry a high risk of human error, which could lead to costly sample re-runs and product release delays. With the repeated pipetting movements common in manual BET, RSI is a major issue, risking personnel downtime and significantly impeding the efficiency and productivity of the QC laboratory.

Moreover, with manual execution of BET, information on samples, assays, and results may not be automatically captured in LIMS. Instead, this is often recorded and entered manually, which can lead to incomplete and erroneous sample and process metadata. Such metadata are essential to streamline regulatory auditing, trending, and troubleshooting.

Challenges With Generic BET Automation

With a clear industry need to overcome the many challenges of manual sample processing, several automated solutions for BET have been developed. Yet, to date, the majority of these have been tailored to a single workflow, making them rigid and suitable only for a small subsection of the endotoxin testing sector – namely end-users that process large volumes of similar sample types. For most QC laboratories, this lack of flexibility has made these solutions challenging to adopt. Consequently, generic automation solutions haven't provided viable industry-wide alternatives to manual BET processes.

The critical underlying issue with these static or rigid automated solutions is that the robotic control code is predetermined and must be changed to accommodate modifications. Therefore, any situation requiring changes to the existing laboratory workflow demands re-writing of the platform's automation scripts. This is not only time-consuming and prone to errors, but also calls for expert programming skills.

An obvious example of where such challenges could arise is when new therapies or devices are introduced into the manufacturing pipeline. Here, new raw materials will be used, and different in-process and product samples will be generated. But, owing to the rigidity of generic BET automation platforms, running the new samples together with existing ones in the same laboratory would either call for regular re-writing of scripts (if the same automation platform is to be used) or investment in a second instrument dedicated to the new procedure. Even if the same test type can be used for the new therapies or devices, it is very likely that a different dilution scheme or number of samples would be required in the platform set up.

Introducing novel test type options presents similar difficulties. QC departments should evaluate alternative methods regularly, both to keep abreast of potential improvements to their processes and mitigate the risk of delivery delays or product quality issues. Notably, the frequently used LAL tests are based on components of animal origin that may be vulnerable to supply shortages and exhibit lot-to-lot inconsistencies due to the biological nature of the lysate. Alternative,

synthetic products based on recombinant Factor C can offer a more sustainable alternative and have been accepted by several global regulatory authorities. Importantly, users must still demonstrate equivalence of this new method to traditional tests for their particular set of samples. Comparing results and performance would, again, necessitate a costly new platform (with associated validation and maintenance) or resource-intensive switching between the existing and alternative assays on the same rigid liquid handler. Ultimately, this hinders the ability of QC microbiologists to explore new technologies that could increase the efficiency and sustainability of their laboratory.

Defining the Ideal Automated BET Workflow

So, what is needed for broader industry adoption of automated BET? First of all, automated solutions mean a significant investment, so ideally, a single instrument should be flexible enough to handle all types of BET, instead of users requiring a dedicated instrument for each workflow. It should also come with user-friendly software that does not require in-depth training and should allow full and simple integration with existing LIMS.

The list of robotic commands needed for full BET automation is extensive and varies with each type of assay (Table 1). To completely overcome the challenges of manual BET workflows, each step of the process should be fully automatable, reducing hands-on time to a minimum. Automated solutions should also cover the entire spectrum of sample types, and even highly complex workflows with subsequent dilution procedures and different diluents or additives should be automatable, allowing laboratories to eliminate manual processes. It should also be possible to analyze complex samples together with standard ones on the same plate, or run different assay types in parallel for straightforward method optimization and new assay evaluation.

Table 1. Robotic commands needed for full automation of BET assays

Sample dilutions
Placing samples on the plate
Placing water blanks on the plate
Creating dilutions for standard curves
Placing standards on the plate
Adding Positive Product Control (PPC) spikes to the correct wells
Moving the plate to the reader for pre-incubation
Preparing the reagents
Moving the plate between the reader and the on-deck incubator
Adding reagents to wells
Moving the plate back to the reader for processing
Reading the plates and processing the results

In-Built Flexibility – The Solution to Current Challenges

Novel, flexible BET automation platforms are now being introduced to address these requirements. Here, users can select one or several templates that specify information about the samples and assay type. Robotic scripts are then automatically generated to perform the automated BET run – without the need for further user intervention. For QC laboratories, this means manual input is minimized, end-user training is simplified, and expert programming or script-writing skills are no longer needed.

By creating a deck layout map that displays all required components and their locations, the software modules in these next-generation platforms enable easy and error-proof setup of the worktable, where the correct positioning of reagents, labware, and consumables are subsequently verified. This confirmatory step ensures an assay cannot commence unless all labware is in the correct position, safeguarding proper assay execution. What's more, the resulting plate layout can be flexibly adapted to the selected template, and users may save the software templates for re-use or create new, unique templates for each assay.

In contrast to generic automated liquid handling solutions, newer flexible platforms can generate the commands necessary to fully automate most BET workflow steps – robotic arms pick up tips, prepare standards, samples, and reagents, dispense them to cartridges or plates, and place the samples in a suitable reader for analysis. As a result, users are free to walk away and focus on other tasks, such as results analysis.

A broad variety of liquids – even those with high viscosity – can also be handled, configured, and tested on these systems. Volumes of sample and reagent liquids required for the selected template are determined automatically. Similarly, diluents and additives are configurable and specified on the template, and multiple dilution steps with different diluents are supported, making manual sample manipulation obsolete.

With automation of a greater number of previously manual steps, there is more comprehensive metadata capture. This data, along with results information, can then be directly fed into LIMS for seamless documentation and full sample traceability. The result is better data integrity, strengthened compliance with standard operating procedures and regulatory protocols, and easier audits – all while providing a completely paperless solution.

Through these capabilities, for the first time, BET laboratories handling a complex variety of samples can access the well-understood benefits of automation. Optimal sample accuracy and precision can be achieved with continuous and high throughput, and the resulting operational efficiency gains mean laboratories can better ensure the commercial viability of their activities. To anticipate the true impact that advanced automation solutions will have on this sector, we only need to look at how advanced, flexible automation has transformed other industries, such as manufacturing, where significant gains in productivity have been realized while simultaneously minimizing waste and unlocking greater employee potential.

Looking Toward Future-Proofed BET

Method reproducibility, accurate data, and sample traceability have become indispensable to meet the high levels of quality needed to ensure patient safety and secure timely delivery of products to market. But the industry is also rapidly changing.

With an increasing focus on developing innovative biotherapeutics that carry a higher risk of endotoxin contamination compared to chemically synthesized, small molecule drugs, the need for accurate and reproducible BET technologies continues to grow. It is unlikely that the capacities of manual procedures will be sufficient to address future demand. In addition, animal-sourced LAL tests may not always be suitable for a variety of reasons, including sample type or sustainability demands, increasing the necessity to explore and use other solutions.

Flexible automation systems that are easy to use and can accommodate new products, raw materials, and assays inevitably help future-proof QC laboratory workflows; not only do they make new method use and evaluation easier, they also empower laboratories to quickly respond to larger scale and higher throughput needs, as well as new regulatory requirements or industry testing changes.

The transition from rigid to novel, flexible approaches heralds a new era of efficient, robust, and accurate high-throughput BET automation for greater laboratory productivity. Ultimately, it will change the way laboratories perform bacterial endotoxin assays, overcoming the challenges and shortcomings of manual processes while better fortifying laboratories against the pace and change of a highly dynamic industry.

About the Author

Ruth Noé is Senior Product Manager for Lonza Bioscience, supporting Bacterial Endotoxin Testing Automation and Software products. She joined Lonza in 2001 as a Product Specialist and later transitioned through roles in Sales Management and Product Support before commencing her current role. Prior to Lonza, Ruth was a Business Development Manager for Covance Laboratories, working with the Biotechnology team. Her previous roles were laboratory based, using RT-PCR in predicting Neuroblastoma relapse in children for the Candlelighter's Laboratory at St James' University Hospital, Leeds, UK, and in virology diagnostics for Public Health Laboratories UK.