Lonza
Pharma & Biotech

The PyroTec™ Robotic Handling System Evolution — Automating the endotoxin test

Summary

For over 10 years, Lonza has been providing customers working with high sample throughput with a semi-automated solution for performing endotoxin assays. This white paper will examine the history, systematic approach and success of the first generation PyroTec™ Liquid Handling System implementation at a dialysis customer site. We will also explore the transformation of the first generation PyroTec™ System into a fully automated "walk-away" solution for customers with the release of the second generation PyroTec™ PRO Automated Robotic Solution.

Lonza has been delivering customer-driven solutions for inefficiencies found in high-throughput dialysis laboratories (i.e. 1,600 samples daily) with PyroTec™ Robotic Liquid Handling System for over 10 years. Our systems are recognized as being the first in the industry, having been utilized by customers during a time when the cartridge systems from our competitors were in the infancy stages of development.

The first generation of the PyroTec™ System — proven success

Table 1 shows 2015 data obtained from a customer who implemented the first generation, large-scale PyroTec™ Automated Solution. Although this customer utilized 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 Lonza robotic solution. The system was a cutting edge solution in the endotoxin industry at the time and is utilized by over 90 % of the dialysis industry today. Of the over 25,000 standard curves that were set up at this customer site, 99.67 % of them passed, with an average % CV of 1.07 %, effectively demonstrating the results obtainable with robotic pipetting. The customer tested over 500,000 samples during 2015, which included samples and samples + positive product controls, with a passing rate of over 97 % and achieving a 1.19 % CV (for samples alone).

Liquid handling robotics instruments are able to perform many of the pipetting tasks currently performed by laboratory staff and do so with greater precision and flexibility. Utilization of these technologies increases technician productivity, reduces lab data errors, eliminates pipette-related repetitive motion injuries and offers potential throughput increases. Lonza's in-house software development experts have written scripts specifically detailing robotic control code in order to process hundreds of plates daily with minimal intervention.

	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%	

Table 1
Customer data demonstrating a large-scale implementation of a robotic pipetting solution

The next generation system — PyroTec™ PRO Automated Robotic Solution

Building on the success of our first generation PyroTec™ System, we have developed the next advancement in endotoxin testing - the PyroTec™ PRO Automated Robotic Solution. PyroTec™ PRO integrates with our WinKQCL™ 6 Software through a patent-pending dynamic control engine for user-selected template configuration (e.g., data transfer, elimination of paper forms, automation of manual data transcription, etc.).

The PyroTec™ PRO Solution further optimizes and automates the routine, manual tasks of endotoxin testing and can improve overall lab efficiency and productivity, while helping ensure data integrity compliance.

Figure 1
PyroTec™ PRO Fully Automated Robotic Solution

A closer look at testing challenges in 2008 — customer case study

Prior to introducing the first generation PyroTec™ Automated Solution, below are real life examples of challenges from a dialysis customer testing 1,600 samples a day.

The customer workflow had a demand of 80 plates (1,600 samples) per day, usually run over two shifts, starting at 8am and ending at 2am the next morning, 6 days a week. A combination of a high sample volume and a very manual process presented many challenges compared to our customer's current workflow.

Turnaround time. Throughout the industry, whether testing dialysis samples, in process samples, or end-product release tests, result turnaround time is of critical importance. While same day results were desired, the turnaround time was often as long as 2 days, something that our customer wanted to improve. As thousands of samples were sent to our customer for testing each day, clearing the lab-testing queue at the end of each day kept the lab from backing up and having even longer turnaround times.

Sample mix up. Because samples were manually sorted and their associated laboratory information system (LIS) accession numbers manually scanned and tracked, the possibility for mixing up samples relative to their position in the list in a test and having results mismatched from the LIS electronic record was a true risk for the lab. Accurate tracking depended on people not making mistakes in the sorting and scanning of the incoming samples.

Mispipetting. All labs suffer from the occasional mispipetting, whether this is pipetting into the wrong well or pipetting the wrong volume. This is a common occurrence with new technicians in all labs, and becomes a lower risk as experience of the technician increases, but the risk never disappears. Mispipetting of a sample can result in the retesting of individual sample or an entire plate, depending upon the mistake made.

Manual data entry. Entering testing result data back into the customer's LIS systems was one of the biggest challenges faced by the QC lab team. Often the manual data entry process would still be going on the next day until 11am.

Retest rate. A common saying in the endotoxin industry is that the most expensive test is the retest. Retesting of samples requires manual intervention, an OOS (Out of Specification) investigation to understand why the sample must be retested. Often times, a deviation is required in order to retest and accept the new results. It is a shared challenge and a metric commonly monitored to show a lab's control over their process. Prior to the improvements made in the lab, our customer had a retest rate of up to 5 %, or four full plates every day.

Work environment. The work environment at most testing laboratories is challenging. To keep turnover low and employees happy, laboratory work must be interesting and there must be opportunities for personal growth. With the original customer workflow, technicians were highly focused on a handful of tedious tasks, such as pipetting and data entry, leaving little or no time for more interesting work.

Multi-tasking. Most companies are limiting the number of new hires for laboratory positions. Unless there is significant growth within the business and increased investment at a particular site, the trend is for upper management to pressure laboratories to do more with the resources they have. From the laboratory management point of view, trying to do more with existing staff is a goal echoed throughout the dialysis, pharmaceutical and medical device industries. Our customer faced a growing sample testing demand, with a fixed laboratory space and existing staff working within this space.

The workflow below is a common one for many QC micro laboratories with throughput of 1,000 samples tested daily:

1. Sort samples
2. Batch samples in groups of 20, enough to fill 1 microplate
3. Build worklists in the customer's LIS system by manually scanning sample barcodes
4. Print out worklists
5. Move samples and worklists to testing stations
6. Manually prepare standards
7. Manually prepare samples
8. Add samples and standards to the plate
9. Manually spike samples
10. Create working reagent, 1 plate at a time
11. Add working reagent to the plate
12. Add the plate to the reader and run the test
13. Print the results of each test
14. Manually transcribe into customer's LIS
15. Generate a retest list based upon results entered

Liquid handling automation — developing a more efficient workflow with the PyroTec™ System

The initial phase of the customer's workflow improvement was to implement liquid handling automation. In 2008, when we began working together with our customer, we first looked at the overall workflow. Because the volume of testing had increased to over 100 plates per day, multiple robots were implemented to handle the workload. Robots were configured to handle six plates per run; whereas other robots were configured to handle nine plates per run. Because of the increased number of plates being run at one time and the limited lab space, it was clear that readers could not be coupled to the deck of the robot, and that this would actually slow down the testing process.

The next steps were to optimize the batching process. To fully optimize the robotic scripts, we worked together to batch samples into "workgroups" of several plates. Six plate workgroups were configured for the smaller robot, and nine plate workgroups were configured for the larger robot. Workgroups were created based upon sample types so that dilution scripts could be optimized.

With optimized scripts and larger sample batch sizes in place, the customer was able to simply load the samples onto the deck, 120 or 180 samples at a time and let the robot create and add the standard curve to the plate. The robot then diluted the samples and added them to the plate, adding an in-well spike to the sample PPC wells. While the robot is running the standard and sample preparation scripts, a technician makes a batch of working reagent large enough to accommodate either six or nine full plates. When the robot has completed adding samples to all plates, our WinKQCL™ Software prompts the technician to add the working reagent to the deck of the robot. Once added, the robot adds the working reagent to all plates. As each plate is finished, users then add these plates to the microplate readers.

Standard curve creation

The snapshots of data below compare the standard curves generated manually to those generated automatically in the initial stages of the PyroTectm System implementation.. See Tables 2 and 3 and Figures 2, 3 and 4.

Run	y-Intercept		Slope	
	Automated	Manual	Automated	Manual
1	3.0378	3.0194	-0.2398	-0.242
2	3.0078	3.022	-0.2475	-0.252
3	3.0204	3.0262	0.2401	-0.234

Table2
Standard curves created by the robot are nearly identical to standard curves by a skilled technician.

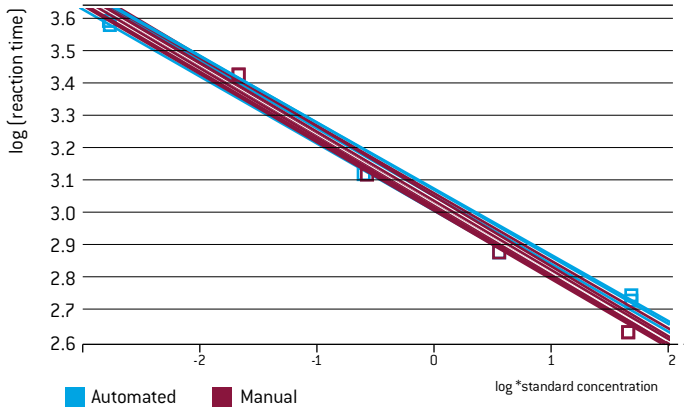


Figure 2
Standard curves generated over 12 assays, manual and automated

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	1,374	1,377	±39	±28	1.8%	1.1%
0.05	260	2,576	±77	±79	1.1%	2.2%
0.005	3,904	3,991	±166	±213	2.4%	1.4%

Table3
Reaction time summary over 12 assays

Means and Standard Deviations			
Method	**#Assays**	**Mean Back.Prediction**	**Std. Dev.**
Automated	12	0.092	0.0156
Manual	12	0.108	0.0158

Table 4
0.1 EU/mL Control Sample

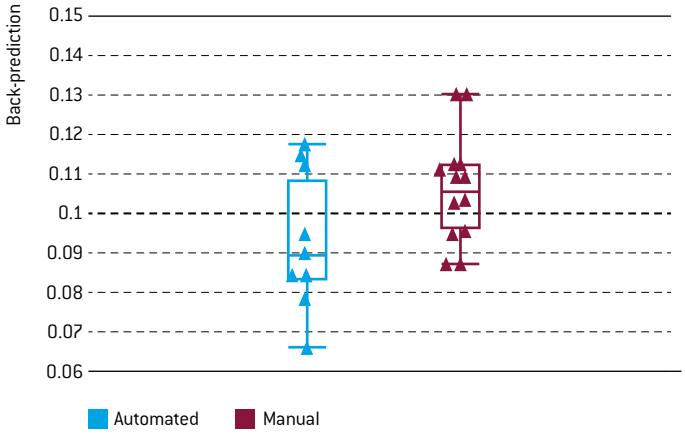


Figure 3
Endotoxin concentrations back-prediction

Means and Standard Deviations			
Method	**#Assays**	**Mean Back.Prediction**	**Std. Dev.**
Automated	12	1.01	0.172
Manual	12	1.18	0.146

Table 5
1.0 EU/mL Control Sample. Automated (robot) versus manual (skilled technician) linear regression back-predicted results for known endotoxin concentrations are nearly identical.

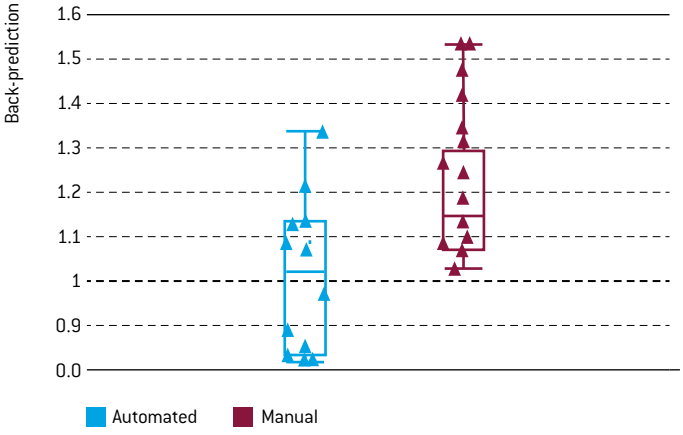


Figure 4
Endotoxin concentrations back-predictions

Data automation — huge time savings realized through an end-to-end WinKQCL™ Software / LIS integration

According to our customer, the biggest improvement made to their process was the time and cost savings realized with the integration of WinKQCL™ Software with the customer's laboratory information system (LIS). While many customers integrate WinKQCL™ Software via the automated data export capabilities of the software, WinKQCL™ Software is also able to import data. On the front end of the testing process, Lonza engineers developed a way to import LIS data through the barcode on the labels of the samples. By adding barcode scanners to the robots, the robots would start the testing process by scanning the barcodes of the samples and then marrying them to the samples defined in the templates in the WinKQCL™ Software. This import process alone saved countless hours by automating the manual barcode scanning task and eliminated the mixing up of samples in the LIS that can occur by accidentally scanning tubes in the wrong order or loading the tubes in the robot out of order.

On the back end of the testing process, the customer's technicians spent even more time manually entering data into their LIS system. Using the automated XML export capability of WinKQCL™ Software, custom drivers were created by Lonza and the LIS vendor to allow sample results to be automatically entered into the LIS database. Rules were then defined to verify the standard curve was valid, the sample result, % PPC recovery and % CV all met specification. With these rules in place, the LIS was able to automatically create a retest list for subsequent dilution and testing.

The new workflow

1. Sort incoming samples
2. Batch samples in groups of 120 or 180, enough to fill 6 or 9 microplates
3. Add sample vials to robot racks
4. Robot scans LIS accession IDS, and prepares and adds standards, samples and PPCs to all plates on the deck (either 6 or 9 at a time)
5. User reconstitutes lysate
6. Robot adds lysate to 6 or 9 plates
7. User adds plates to readers
8. WinKQCL™ Software runs the tests and automatically exports data to the laboratory information system
9. LIS creates retest list

With such an improved workflow, the customer certainly realized hard savings: costs savings, revenue growth, and time savings. In addition, many soft savings were attained as well, for which exact numbers are more difficult to report, but which any testing lab can certainly appreciate.

Hard savings

Time savings. Across all aspects of the endotoxin testing process, the customer realized an estimated 8 full time employee shifts, or 64 hours per week, savings in labor.

Cost savings. Significant savings in time translated into dollars saved. A reduction in retesting samples to 1.1 % in 2014, and below 1 % in early 2015, was realized.

Top line growth. As a more efficient laboratory, the customer began delivering high quality results to their customers faster.

Soft savings

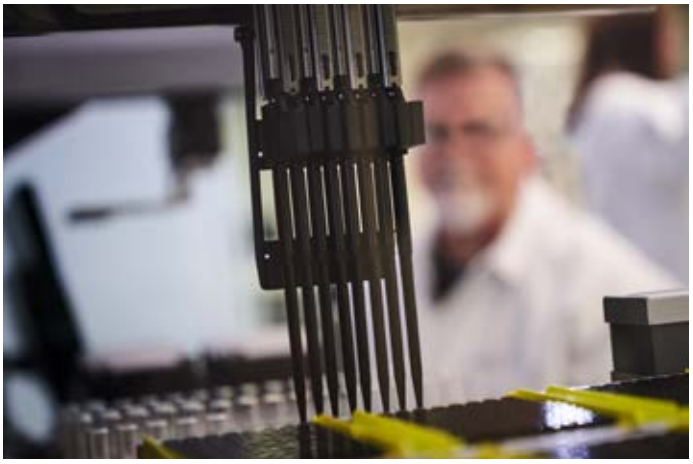
Ergonomics/safety. Users could now spend significantly less time pipetting and typing. Ergonomics is an aspect of safety, which the customer and its employees value.

Competitive edge. The customer could turn around results within hours of receiving customer samples. With faster turnaround times and the ability to absorb a higher volume, they are in a better position in the dialysis market.

Happier employees. In the past, laboratory technicians were only doing what classifies as technician "work". The customer now has employees that have the time to learn and engage in numerous new roles and activities beyond their technician roles. Employees who were once only involved in endotoxin testing are now involved in process optimization projects, sterility testing, chemical analysis, and other interesting sample processing tasks.

The next advance – the introduction of the PyroTec™ PRO Automated Robotic Solution

Because of the increasing demand for automating the monitoring and testing for all laboratories' requirements, we have created the PyroTec™ PRO Automated Solution integrated with WinKQCL™ Software Version 6, which facilitates the development of an automated system while requiring minimal manual steps for performing endotoxin assays. The PyroTec™ PRO Solution also provides performance that is equivalent to, or better than, the performance of assays that are manually executed by an experienced technician.



PyroTec™ PRO is the next advancement for automated endotoxin sampling (See Table 6). It accurately generates standard curves and easily handles complex dilution schemes. The simplicity of the proprietary software algorithm 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 PyroTec™ PRO Solution is balanced with the demonstrable accuracy of robotic pipetting in large-scale or small-scale formats with all liquid classes. As seen with customer data, the consistency and accuracy of both standard curves and samples in a robotic environment is formidable in both quality and quantity.

PyroTec™ Liquid Handling System	PyroTec™ PRO Automated Robotic Solution
Semi-automated	Fully automated walk away solution
Script development for new assays in Freedom EVOware®	Dynamic script writing within WinKQCL™ Version 6 Endotoxin and Analysis Software
High throughput with up to 9 plates per run	Small – medium output with 2 plates per run
Simple dilution schemes	Complex dilution schemes with ancillary buffers
Basic liquid class	Liquid class to handle complex samples
Supports Lonza's Kinetic-QCL™ Chromogenic Reagent	Supports Lonza's PYROGENT™-5000 and Kinetic-QCL™ Assays with PyroGene™ rFC Assay coming soon.

Table 6
First generation PyroTec™ Liquid Handling System and advances made with the new PyroTec™ PRO Automated Robotic Solution

PyroTec™ PRO Robotic Solution lays the foundation for a fully automated endotoxin testing platform. It offers complete interconnectivity with a variety of client databases, which can 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.

Conclusion

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

For high-throughput laboratories that want to move away from a manual benchtop workflow and reduce errors and retests, our PyroTec™ PRO Robotic Solution integrated with our WinKQCL™ 6 Software offers a fully automated solution that enables better control of the entire endotoxin testing process by removing the potential for human error while enhancing lab efficiency and productivity.

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