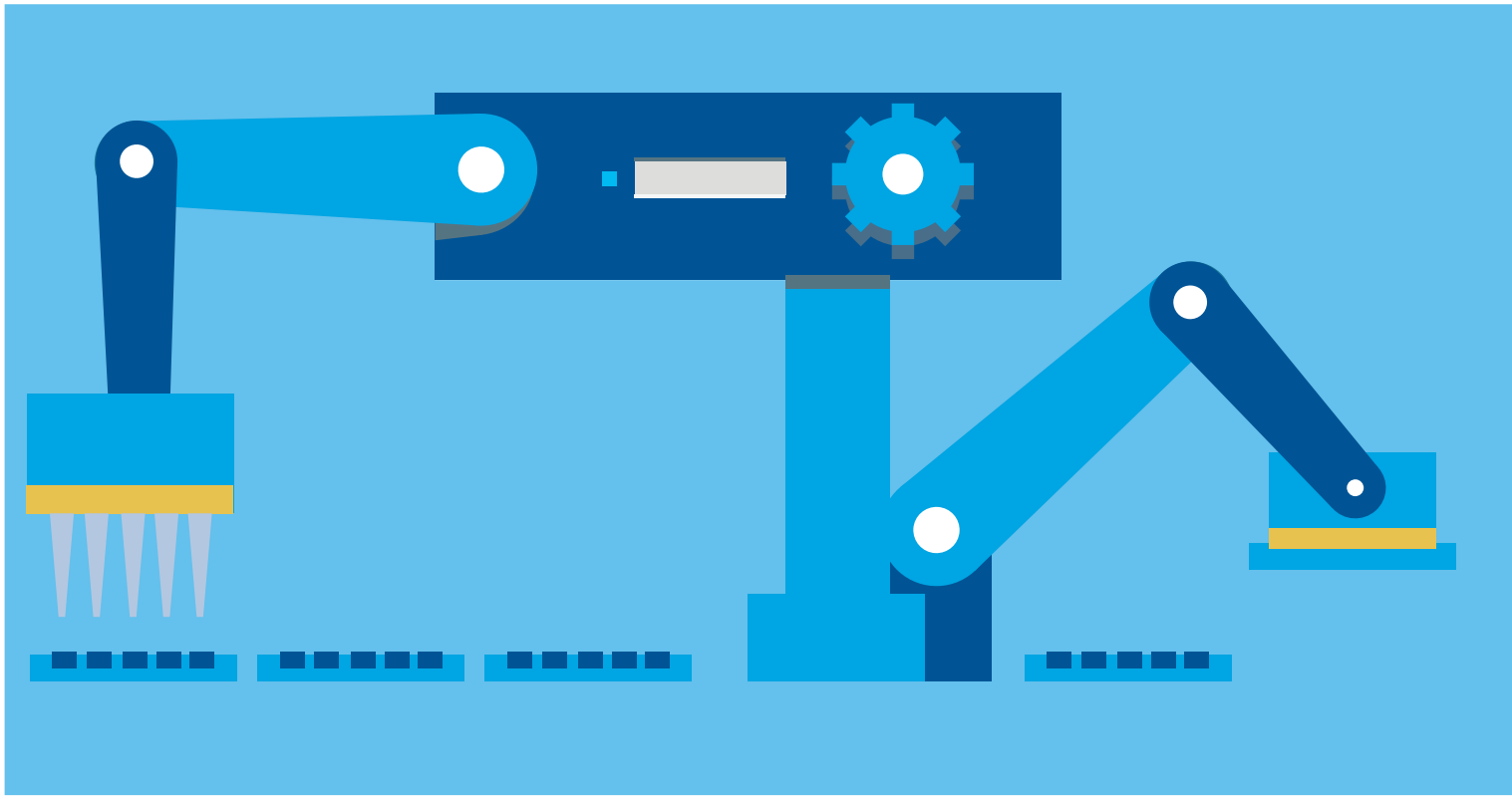
Bioscience Solutions

PyroTec™ PRO Robotic Solution for Endotoxin Detection

FAQs Tech Tips



Move from Manual Benchtop Processes to an Automated Workflow

PyroTec™ PRO Robotic Solution is a new automated, plate-based system that offers users the ability to move away from manual benchtop processes associated with endotoxin tests. The PyroTec™ PRO System integrates with WinKQCL™ Software, achieving high-throughput sampling in three simple steps, offering an automated workflow for high-volume QC labs.

This Tech Tip will help users gain more insight into what is assay automation and how it can transform a busy manual-based endotoxin laboratory into a streamlined, automated system. Topics include: sample dilutions, integration of laboratory information management system (LIMS), automation processes workflow with WinKQCL™ Software, deck setup and assay runs. Technical specifications for PyroTec™ PRO Robotic Solution are also provided.

What is assay automation?

Assay automation is a custom solution comprising software, hardware, integration and engineering to reduce or eliminate manual intervention in the performance of an assay. This constitutes a comprehensive look at your laboratory environment, an analysis of strengths, weaknesses, specific pain points, and a determination of how automation can be introduced to streamline and improve performance, and through process optimization, can result in time savings and error reduction.

Can you provide a short overview specification sheet on PyroTec™ PRO Robotic Solution?

Yes, please see table below.

Description	Answer
Throughput per run	**42** (21 per plate in duplicate w/ duplicate PPC) with five point standard curve with two negative controls.
Sample handling	**Yes**
Prepares sample dilutions	**Yes**, 128 (includes sample tubes). All 128 tubes are available to make simple to complex dilution schemes.
Prepares auxiliary dilutions	**Yes**, for dilutions requiring other than liquid reagent water, i.e. Beta-G blocker.
Reconstitution of RSE/CSE	**No**
Vortex of reconstituted RSE/CSE	**No**
Prepares endotoxin standards	**Yes**, flexibility to make up to eight standards per assay (most common is five).
Prepares positive product controls	**Yes**
Prepares negative controls	**Yes**
Incubate plate	**Yes**
Reconstitution of LAL reagents	**Yes**
Pipettes LAL to plate	**Yes**
Places plate in incubator	**Yes**
Barcode scanner	**2D barcode**; various standard formats supported. Barcodes are entered on the template in WinKQCL™ Endotoxin Detection and Analysis Software as well as adaptability on the robot deck.
Sample tubes type	**Pyrogen-free USP Type 1**, 13 x 100 mm tubes with max volume of 8 mL per tube (128 tubes available).
Ancillary accessories	**Tips, reagent reservoirs, and microplate** supplied by Lonza certified down to 0.005 EU/mL.
Deck setup time	**Fully loaded plate**: 5 minutes minimum.
Preparation of plate	**Fully loaded plate**: 30 minutes minimum; after LAL load: 43 minutes.
Runtime in reader	**1 hour**
Total time to result including preparation and run	**1 hour 50 minutes** for 21 samples.
Sample test volume	**100 µL**
Method supported	**Kinetic chromogenic**; kinetic turbidimetric available soon.

Is it possible to automate the standard dilutions? What is the state of the endotoxin standard to be placed in the deck? Reconstituted endotoxin? Series of endotoxin dilutions?

The standard endotoxin dilutions are created by the system based on what is specified in the WinKQCL™ Software Template specific for that assay. The system does not reconstitute the endotoxin. The endotoxin must be reconstituted and vortexed offline by an analyst prior to running an automated assay – and then placed on the robot deck. The system uses the reconstituted endotoxin to create the standards.

How does the PyroTec™ PRO System mix samples after sample dilution?

All mixing on the system is done by the liquid handling robotic arm.

Am I limited in the amount and type of sample dilutions?

With 128 spaces available for sample tubes on the deck layout, you are only limited on what you use. You can create a multitude of sample dilutions with different sample types; utilizing liquid reagent water in addition to auxiliary dilutions simultaneously within the same run.

Is there a connection to LIMS (i.e. import and export of sample IDs, worklists, and results)? Can you import data from LIMS?

Yes, you as the end-user can do so to setup and define the trigger for the data transfer. Lonza offers services to assist with this.

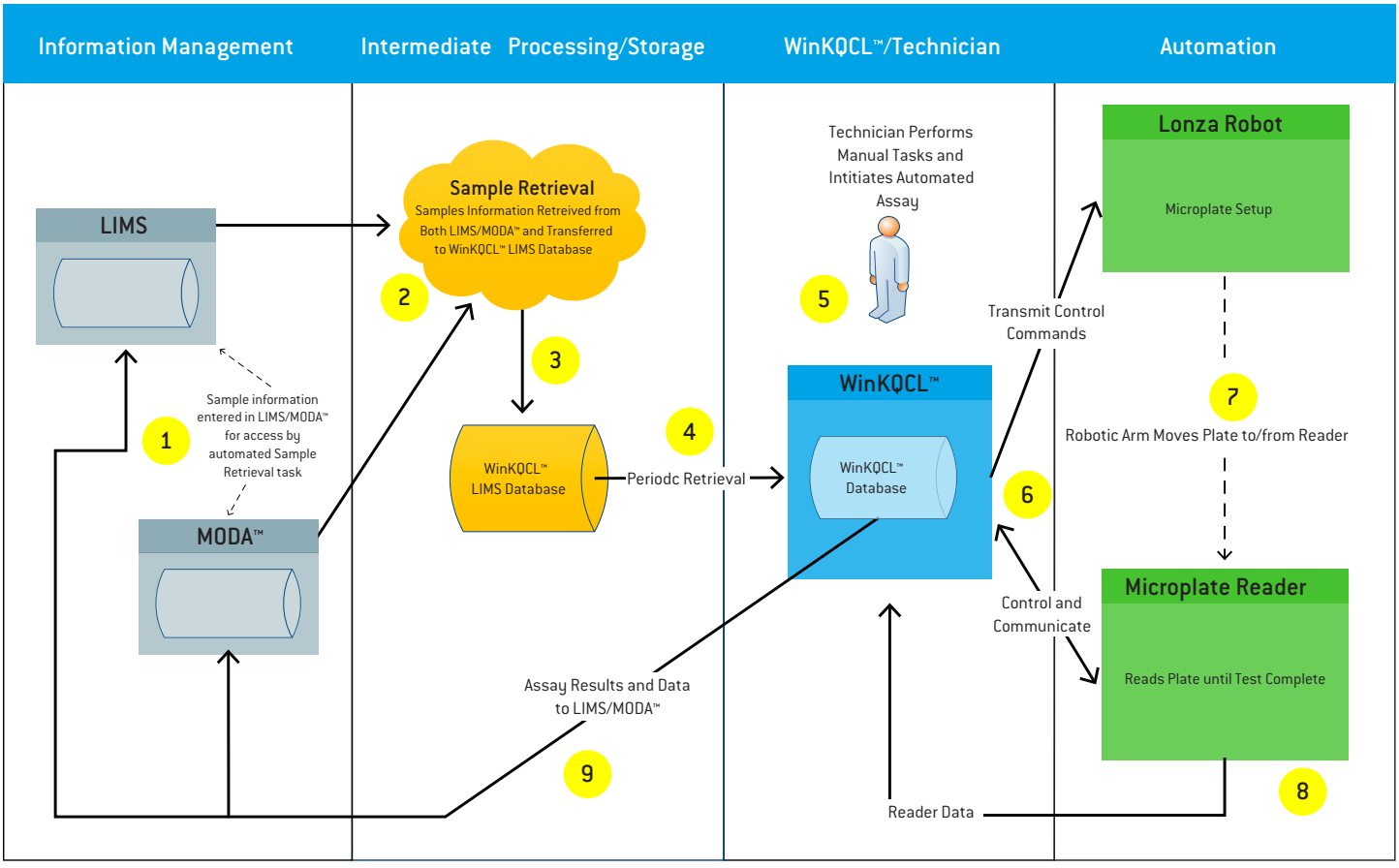
What about dilutions and dilution factors? Could this information be transferred to WinKQCL™ Software if contained in a LIMS?

Yes, this can dynamically create the dilution scheme in WinKQCL™ Software. Products also known in the WinKQCL™ Software will have their own liquid class identified.
The dilution factor is specified on the template. This information can be pulled from LIMS if available. For example, an export created in CSV can be exported back into LIMS.

Can we do all operations within a LIMS?

Theoretically, yes, as all have simliar API/interface systems. Lonza can work with you to assist in the integration.
Please see flow chart on next page as a reference to a high-level process overview.

Endotoxin Automation High-level Process Flow

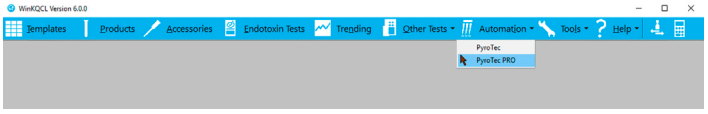


What manual steps are controlled by software?

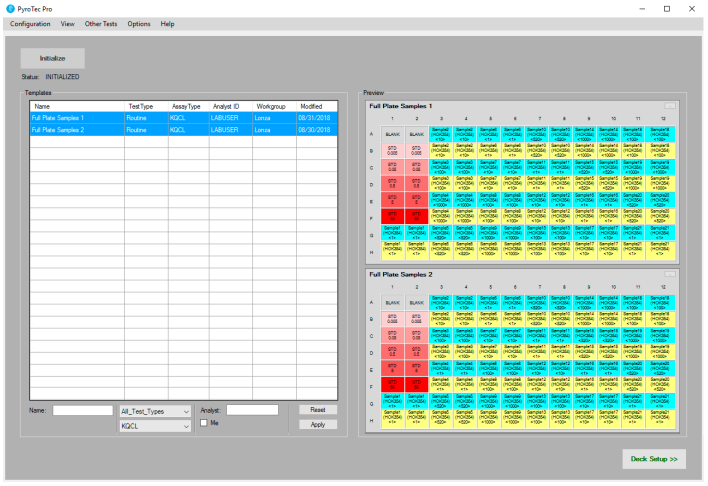
Pop-up windows guide you through manual preparation (endotoxin, LAL, sample), and every step has to be actively confirmed by the user, which acts as a fail-safe. For data integrity purposes, it reduces the risk of operator error in setting up the deck.

The endotoxin automation high-level process workflow on this page shows the process in the automation module software, a fairly simple process. A user manual will be included in the purchase of the PyroTec™ PRO System.

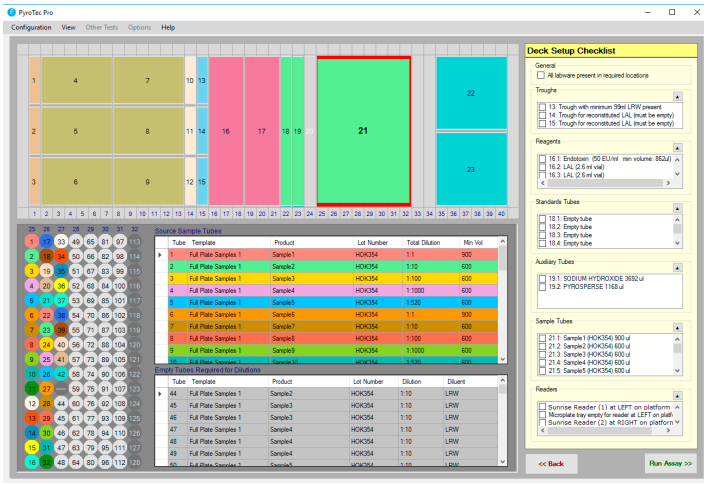
Step 1: Initiate the automation module within WinKQCL™ Software



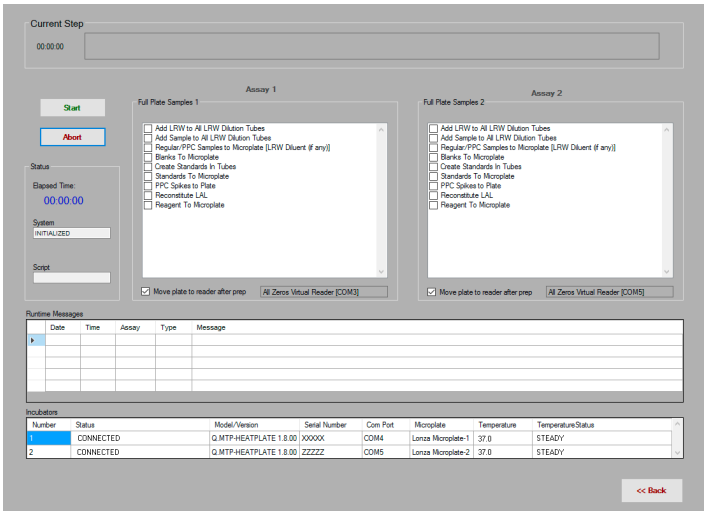
Step 2: Select the template(s)



Step 3: Setup the deck per the instructions provided



Step 4: Initiate the automated run



Endotoxin

The endotoxin must be manually reconstituted and vortexed by the analyst – the same as would be done to perform a manual assay. After the analyst vortexes the endotoxin, the analyst must then place the endotoxin on the deck at the location specified in the deck layout instructions (Step 3).

LAL

The robot reconstitutes the LAL reagent. The user is only required to place the open LAL vials at the locations specified in the deck setup instructions (Step 3).

Sample

Sample tubes must be placed at the locations (and volumes) specified by the deck setup instructions (Step 3). When barcode scanning functionality is implemented, the samples may be placed anywhere in the sample tube racks.

Deck setup

The deck setup interface (shown in Step 3) provides detailed instructions regarding where components must be positioned, and what volumes of liquids must be present. Notice that there is a checklist to the right of the interface. The analyst must check off every item in the check list to confirm that all positions and all volumes are correct. The software will not allow the automated assay to be run until all items in the check list are confirmed.

How does liquid level detection work if different sample containers are used (conductive or pressure based?)

The system currently only supports samples in 13 x 100 mm tubes. Conductive liquid detection is used. If a customer wants to use containers other than 13 x 100 mm tubes for samples, a special configuration would be required.

Are all the consumables endotoxin-free?

All consumables including 13 x 100 mm tubes, pipette tips, reservoirs and microplates used in the system are certified to be endotoxin-free down to 0.005 EU/mL.

Can you run different samples (different treatment, dilution etc.) on one plate?

The system can accommodate a variety of samples on one plate with different dilutions applied to each sample. The system will apply whatever LAL reagent water dilutions are assigned to each sample on the WinKQCL™ Software-generated endotoxin template. Each product (i.e. sample) defined in the WinKQCL™ Software Database is configurable in the automation module software to use a specific liquid class, which determines the pipetting parameters to be applied (different liquid classes for aqueous, viscous, foamy, etc). When a new sample in the WinKQCL™ Software Database is detected by the automation software, it is initially assigned a default liquid class, but a different liquid class can be assigned by the user of the system if necessary. The automation software is also able to handle auxiliary dilutions such as beta glucan blocker and Tris buffer. Auxiliary dilutions are configurable per sample. The order in which the auxiliary dilutions are to be applied is also configurable. In addition, the LAL reagent water itself can also be configured as an auxiliary dilution if necessary.

Can you provide more information on the validation of endotoxin resuspending using a pipet vs. vortexing?

The analyst is required to reconstitute and vortex the endotoxin prior to running the assay. However, the robot does reconstitute and mix the LAL reagent. As for the standard curve, the Lonza package insert states that each standard dilution must be vortexed – this can't be done by the robot, so the robot uses up/down mix steps instead. We have study data that confirms the up/down mixing per standard dilution is comparable to using a vortex.

Has any stability testing been performed for the endotoxin and lysate that stay on the instrument before use?

Our data has shown that it is recommended to place the reagents on the deck prior to running the automated assay. This would be similar to the same methodology when performing the assay on the bench. Customers should follow procedures similar to the way that they would not allow reagents to sit on a benchtop for a long period of time prior to using them for a manual assay. For the lysate specifically, it is not reconstituted until just prior to being transferred to the microplate, so for any time spent on the instrument prior to that, the lysate is in powder form.

How many plates can be processed with the instrument?

The system can accommodate up to two independent microplates on a single run of the instrument (i.e. a single deck setup).

What are the limiting factors (reagents, consumables etc.)?

The limiting factor is deck space on the instrument. Currently the instrument only has enough space to accommodate the labware required for two assays.

Is it possible to reload the instrument during a run with reagents, consumables, samples? Can you continuously load? Is an automatic retest supported?

After the last microplate is placed in the reader and control is transferred to WinKQCL™ Software to begin reading the plate, the instrument's work is complete. At that time, it would be possible to start preparing the deck for the next run. However, the next run should not be started until all microplate readers (either 1 or 2) have completed reading the microplates, and all microplates have been removed from the readers by the analyst. When two assays are performed in a single run, the assays are performed sequentially, not concurrently. The instrument will not start the microplate setup for the second assay until the instrument has placed the microplate from the first assay in the reader to be read by WinKQCL™ Software. When WinKQCL™ Software begins reading the first microplate, the instrument will begin processing the microplate setup for the second assay. The system does not support an automatic retest of a failed sample. The analyst would need to add the sample to the next run to retest it.

Runtime should be fitted to a normal lab-workday. In best case after 8–9 hours, can an overnight run be started?

Typical runtime from start of the automated microplate setup to completion of WinKQCL™ Software reading the plate for a single assay is 1.5 hours – 2.5 hours, depending on the number of samples and the complexity of the dilutions. Therefore, a two-assay run will typically complete in 3 hours based on the simplest testing scheme. The second run can begin setup and preparation while the first plate is being measured.

Can a second/third plate be prepared while the first plate is measured?

Yes, after the instrument places the last microplate of the current run in the reader and WinKQCL™ Software begins reading the plate, deck-setup for the next run, which can be either 1 or 2 assays, can begin. However, the next run should not be started until WinKQCL™ Software indicates that the last assay (either 1 of 1 or 2 of 2) has completed, and all microplates (1 or 2) have been removed from the readers by the analyst.

Can the Pyrotec™ PRO System be utilized with the PyroGene™ rFC Assay?

The system currently only supports the Lonza KQCL assay – but support for the PyroGene™ rFC Assay (and PYROGENT™-5000 Assay) is planned for an upcoming system release.

Are there any accessories that need to be purchased to run PyroTec™ PRO Robotic Solution other than standard reagents, dilution tubes, and microplates?

Yes, pipette tips and troughs will be needed, will be supplied by Lonza and are certified to be endotoxin-free down to 0.005 EU/mL.

How long does it take to perform the installation, operational and performance (IOPQ)?

There are many components that need to be installed and validated. Depending on the customizations needed, estimated time for installation is 2 weeks.

Can you provide more information on the IOPQ?

1. Installation Qualification (IQ) – ensures that the hardware and software are installed and configured properly in preparation for further testing. Basically, this verifies that the software is installed and that readers and incubators have been configured correctly.
2. Operational Qualification (OQ) – ensures that hardware and software components are in proper working order (confirms functionality of LiHa and RoMa arms and functionality to transfer microplates to/from readers).
3. Performance Qualification (PQ) – evaluates the overall functional performance of the system by performing actual automated endotoxin tests using Lonza endotoxin testing reagents (currently plan to run an assay (3x) that uses complex dilutions).

Is the PyroTec™ PRO System compatible with other software like Charles River EndoScanV™, non WinKQCL™ Software?

The system is compatible only with Lonza's WinKQCL™ Endotoxin Analysis and Detection Software.

Our lab would benefit from utilizing the PyroTec™ PRO Robotic Solution, what would we need to do prior to scheduling an install?

The best next step would to be to contact your sales representative to schedule a meeting to discuss:

- Building use requirements
- Map sample workflows
- Identify launch sample types
- Build cater validation/qualification procedures towards use case
- SOP revisions/assistance

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