

e-book

BET Automation Simplified

How to achieve simple,
automated endotoxin testing



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Introduction

Automation in today's QC environment

To succeed and thrive in today's pharmaceutical manufacturing environment – particularly in the quality control (QC) lab – it is critical to increase efficiency while maintaining compliance. The need to quickly and accurately test samples, review data, and release products drives innovation in analytical instrumentation and software, with the ultimate goal being to deliver medicines to patients safely and efficiently.

While automation and robotics exist to reduce manual labor and streamline processes, most labs haven't fully adopted these technologies due to the complexity of the instrumentation, training, and validation practices that come with them. Thus, many labs still focus their attention on traditional testing, perhaps training “super users” and trying to achieve small time savings through better technician performance. Labs also seek out software to try to increase efficiency and flexibility, while also achieving the latest in data integrity compliance. Unfortunately, many automated technologies and new software either don't meet the needs of users in the QC lab or cannot be implemented and validated without a significant amount of effort. As a result, labs are not realizing the true benefits of automation and are eager for alternative solutions that are simple and easy to implement and maintain.

Endotoxin testing then & now

Prior to the development of current compendial bacterial endotoxins testing (BET) methods, drug pyrogenicity was measured using the rabbit pyrogen test (RPT). In the 1970's, Limulus amoebocyte lysate (LAL) was approved by the FDA for use as a new, more sensitive, and more consistent detection methodology for pyrogens in drug product. LAL innovation began with the manual gel clot technique, evolved into endpoint photometric methods, and eventually resulted in the popular kinetic photometric (kinetic chromogenic and kinetic turbidimetric) methods. Most businesses today are using the compendial kinetic chromogenic or kinetic turbidimetric BET methods.

Since the 1980's, there have been nominal improvements for most QC labs when it comes to their everyday experiences with compendial endotoxin testing. Automation has not been widely adopted due to the lingering need for a system that maintains compliance and accuracy without introducing complexity. Labs are seeking automated solutions that are easy to implement, fast to validate, and provide day-to-day advantages such as ease of use and decreased contamination.

This eBook will explore what automation of endotoxin testing entails and the value of implementing newer technologies and software that are simple and straightforward for QC labs to deploy. It will demonstrate how to implement simplified endotoxin automation in your organization – from technology assessment and automation of assays, to validation, compliance, and software.

What are Ways to Automate Endotoxin Testing, and What are the Challenges?

There are often many goals associated with moving to an automated platform in the QC lab – time savings, fewer errors, process improvement, better integration with various systems and software, and others. It is important to think about the ultimate goal and what that destination looks like when considering ways to automate. Doing so will help determine which technologies to choose and which pathways you may want to take or avoid.

Keep your destination in mind

Automation of endotoxin testing can be achieved using technologies such as microfluidics and/or robotics. Pipetting, liquid handling, and mixing of reagents can all be automated, along with preparation of standard curves and positive product controls (PPCs). Software is also an essential component of an automated system in today's lab and should be considered as you define your ideal destination.

What are ways to automate endotoxin testing?

- **Automation of liquid handling:** With 200+ pipetting steps required to run a traditional LAL assay, it's no surprise that automation of liquid handling is a primary goal within endotoxin automation. Whether using robotic or microfluidic liquid handling, these technologies minimize the hands-on time needed to repeatedly and consistently deliver accurate measurement and dispersion of liquids. Additionally, potential human errors and demands on analyst training and performance are greatly reduced.
- **Automation of standard curves and PPCs:** Technologies that provide preloaded endotoxin standards or reagents allow users to take greater advantage of time savings, simplification, and

reduced opportunity for error. However, caution should be taken to ensure that compliance is not compromised. To run a compliant assay, users must construct at least a three-point standard curve in duplicate using standardized endotoxin, have duplicate negative controls, and run each sample in duplicate with a PPC, also in duplicate.

- **Data management & data integrity:** Although aspects of data management are still conducted manually and require human interpretation and approval, the most beneficial platforms will automate as many factors and features possible to ensure data integrity compliance while making data management as efficient as possible. Software is an essential component of today's testing – including data review, sign off, and product release – and should be purpose-built for automated endotoxin testing.

Choosing your path

While robotic liquid handling for endotoxin testing has been around since the 1990s, it has not been widely adopted. Cartridge-based technologies have also emerged to offer easier assay setup. The reduction in hands-on time appeals to lab managers, but concerns remain around validation, maintenance, compliance, contamination, and overall ease of use with some of these platforms. Now, centripetal microfluidic technology is available to automate liquid handling in a platform that is simple to use, compliant, and maintains the small footprint and easy validation and maintenance of a microplate reader. Navigating the route through validation, training, and implementation that easily leads to successful daily use is no longer a challenge.

What are the challenges with robotic automation?

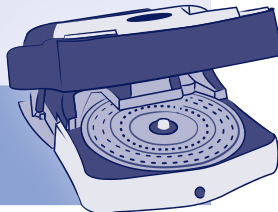
- **Challenges with validation:** The upfront complexity of system setup, installation, and validation with robotic platforms can limit successful implementation. When QC labs are resource constrained, it can be difficult to find time for validation or training that is not extremely straightforward. Other considerations that arise include the need for integration and validation of software scripts, and the potential need for additional hold time studies (reconstituted LAL, standard curve dilutions, open or exposed cartridges).
 - **Challenges with compliance:** Not all robotic liquid handling systems prepare a standard curve or include negative controls. Compendia require both to ensure every assay has controls and accounts for any technician, environment, or reagent variability from assay to assay. And it's good science!
 - **Challenges with liquid handling:** Robotic systems limit the pipetting steps performed by technicians, however analyst time is still required for robotic deck and software setup, and time-to-results is not significantly improved. Additionally, pipetting steps with robotic platforms are not always reduced in preparation of standard curves and PPCs. Similar to running traditional LAL assays with a 96-well plate, the environmental exposure to contamination is not mitigated with robotics. This is in contrast to closed, microfluidic systems that can automate liquid handling while also reducing environmental exposure.
- ✓ **Pulling it all together:** The easiest path to simple, fast bacterial endotoxin testing (BET) is using an integrated, plug and play platform that is purpose-built for endotoxin automation. Ease of use, hands-on time, environmental contamination, compliance, and software can all be addressed to simplify automation.
 - ✓ **Never compromise on compliance.** Be sure requirements are met for standard curves, negative controls, and PPCs. Be confident with your choice of LAL. And don't forget about data integrity and 21 CFR Part 11!

HOW DIFFERENT ENDOTOXIN TESTING PLATFORMS WORK

A Summary of Kinetic Chromogenic Testing Systems

	ROBOTIC PLATFORM WITH 96-WELL PLATE READER	ROBOTIC PLATFORM WITH CARTRIDGE READER
TECHNOLOGY	Robotic liquid handling is integrated with traditional 96-well plates to pipette LAL reagents, control standard endotoxin/reference standard endotoxin (CSE/ RSE), and samples.	A liquid handling robot is paired with LAL cartridge technology. Cartridges contain LAL reagent, chromogenic substrate, and CSE.
STANDARD CURVE AUTOMATION	Yes. Robotic dilution of CSE/ RSE.	No. Archived standard curve. CSE embedded.
HANDS-ON TIME	 Robotic deck layout and script	 Robotic deck preparation and cartridge loading
LAL USAGE		
SAMPLE THROUGHPUT		
COMPLIANT ENTERPRISE SOFTWARE		
VALIDATION CONSIDERATIONS	Robotics and standard IQ, OQ, PQ	Robotics, cartridge hold time study, and standard IQ, OQ, PQ
FOOTPRINT IN LAB		

Based on average 8-hour shift using a single platform.

MULTIPLE CARTRIDGE READER	96-WELL PLATE READER	MICROFLUIDIC AUTOMATION PLATFORM – SIEVERS ECLIPSE*
This multi-cartridge system uses LAL-cartridge technology to run one sample per cartridge. LAL reagent, chromogenic substrate, and CSE are contained within disposable cartridges.	Performing traditional LAL assays with 96-well microplates requires a high volume of pipetting and is time consuming and prone to errors. Standards and samples must be prepared, and lysate must be reconstituted prior to addition.	Microfluidic automation minimizes pipetting and mixing steps without the use of robotics. This microplate-based platform uses embedded RSE with centrifugal microfluidics to automate standard curves, PPCs, and mixing. Minimal LAL reagent is required.
Cartridge based	Manual pipetting	Automated microfluidic liquid handling
No. Archived standard curve. CSE embedded.	No. Manual pipetting of CSE dilutions.	Yes. RSE embedded.
 Individual sample loading and pipetting	 No robotics, extensive manual pipetting	 No robotics, minimal pipetting
		
		
		
Standard IQ, OQ, PQ	Standard IQ, OQ, PQ	Standard IQ, OQ, PQ
		 

*Trademark of Veolia; may be registered in one or more countries. 300 00052 EN Rev. A

What is Microfluidic Automation of BET Assays?

Automation using a centripetal microfluidic platform is the simplest form of BET automation available to the market today. It leverages a network of microchannels to direct and mix fluids to automate endotoxin assays. This is achieved within a small, compact microplate that is analyzed using an incubating benchtop spectrophotometer similar in size and function to absorbance microplate readers used for traditional LAL assays.

Why microfluidics?

Microfluidics has been a thriving research field for over 30 years due to its abilities to meet demands for chemical analyses that are accurate, cost-effective, reliable, and sensitive. It manipulates small reaction volumes within narrow channels, and by doing so can reduce costs and consumption of reagents and samples, decrease time setting up reactions, and increase sample throughput. Exploiting microfluidics for endotoxin testing allows for the

desired characteristics of assay automation – easy liquid handling, reduced reagent consumption, and cost-effective testing without any compromises in accuracy or compliance.

In centripetal microfluidic automation of BET assays, microfluidic liquid handling facilitates accurate and rapid dispersion of BET reagents and samples with drastically reduced volumes of sample and LAL. This is achieved using microfluidic channels, metering chambers, and centripetal force as the microplate spins to control and automate all liquid measurement, flow, and mixing in preparation for analysis. The microfluidic system enables users to carry out the same biochemistry that is performed in traditional 96-well plate assays but with minimal manual effort, greater consistency, and reduced reagent consumption.



Centripetal microfluidic automation of BET assays with the Sievers Eclipse

<i>Does</i>	<i>Does not</i>
– comply with all global compendia	– alter the biochemistry of BET assays
– drastically improve ease of use	– require significant time to set up
– reduce hands-on time, including pipetting	– need standard curve or PPC preparation
– reduce amount of training required	– require a large amount of space or complex equipment
– reduce environmental contamination	– require extra sample hold time studies
– precisely measure and distribute liquids in 1:1 sample to lysate ratio	– require complex validation
– decrease amount of LAL, LAL Reagent Water (LRW), and sample needed	– require significant in-depth training
– utilize commercially available LAL	– require in-depth maintenance
– allow for scalability	– need an analyst to monitor at all times
– make your life easier when it comes to endotoxin testing	– introduce additional complexities

The Benefits of Centripetal Microfluidics for BET Automation

Centripetal microfluidic automation simplifies endotoxin testing by offering a solution that is extremely easy to set up, use, and maintain. It enables labs to achieve the ease of use, ease of training, and high throughput they want, without having to be concerned about compliance, accuracy, complex validations, or footprint. Using this technology, fully compliant endotoxin assays can be set up in as little as nine minutes with a fraction of the pipetting steps typically required.

Preloaded standards and PPCs are used to automate standard curves and PPC spikes, saving labs significant amounts of time and reducing pipetting steps and opportunities for error. In addition, because a closed microfluidic system is used, environmental contamination is reduced. A 1:1 sample to lysate ratio is precisely delivered using this consistent and reliable technology. Overall, with microfluidic automation, compendial endotoxin assays are performed effortlessly, quickly, and with fewer retests.

- ✓ **By the Numbers:** With centripetal microfluidic automation using the Sievers Eclipse, fully compliant endotoxin assays are set up in as little as 9 minutes and less than 30 pipetting steps, with up to 21 samples and up to a 5-point standard curve. Just 1 mL of LAL is required.

Spotlight on Ease of Use: By drastically reducing pipetting steps, centripetal microfluidic automation decreases the complexity of assay setup and mitigates risks and opportunities for errors that lead to costly retests. In addition, microfluidic liquid handling precisely measures all liquids for the end user, which means that the precision typically required during the physical action of pipetting is reduced. With preloaded standards and PPCs, all the technician needs to do is pipette Water for BET, samples, and 1 mL LAL.

See It In Action – Simplified BET Automation



Sievers Eclipse® Bet Platform
Simple Steps to Get Started



Simplified Endotoxin Testing & Software

Sievers Eclipse BET Platform

► **Experience simplified endotoxin testing with microfluidic automation and the latest in compliant software.**



ANALYZER

Absorbance analyzer with consistent incubation control at 37 °C, centrifugal technology, and secure data transmission.

MICROPLATE

Precise liquid handling device that achieves automation through innovative microfluidic technology with embedded endotoxin standards and PPCs.

SOFTWARE

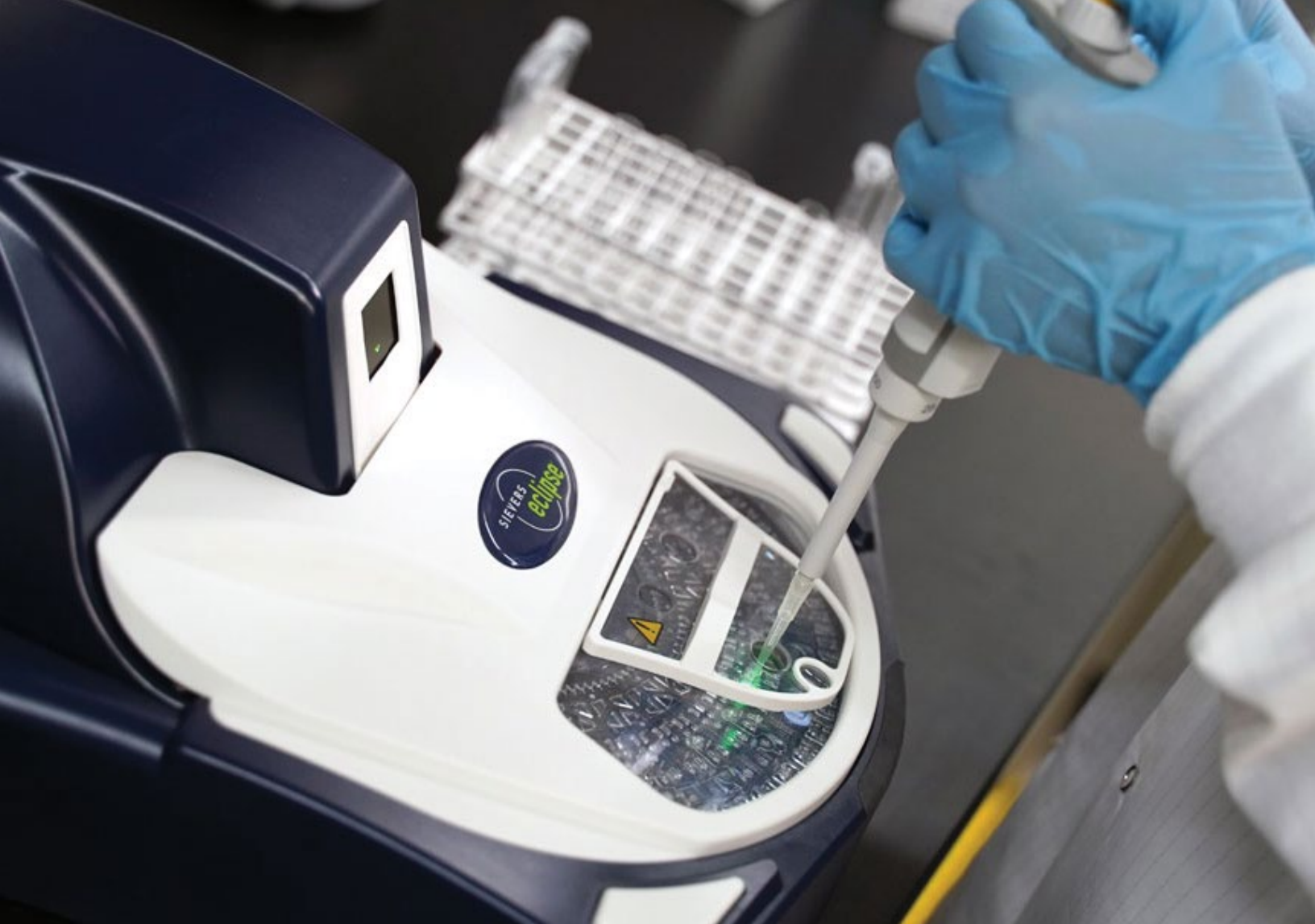
Highly customizable enterprise solution with 21 CFR Part 11 and ALCOA+ Data Integrity compliance features.

How Successful BET Automation Can Positively Impact Your Facility

Successfully implementing a BET automation program should result in more than just being able to test more samples in the QC lab. In most organizations, there are multiple areas of the business that can benefit from thoughtfully designed, automated technologies.

Let's consider some of the areas within a pharmaceutical manufacturing plant that are impacted by the introduction of new technologies:

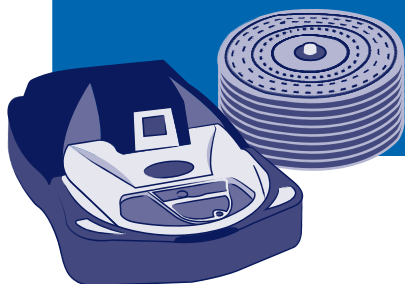
- **Supply chain and storage:** When reagents and consumables are minimized or simplified in terms of storage (e.g., transitioning from cold storage to room temperature storage), this is a win in the warehouse. It's important to keep in mind all of the products and consumables a system utilizes and be sure that supply chain security and storage issues are addressed. For endotoxin testing, cold storage is required for various components of the biological assay including LAL cartridges, however newer automation platforms introduce the ability to significantly reduce cold storage of LAL and eliminate cold storage of Reference Standard Endotoxin (RSE)/ Control Standard Endotoxin (CSE) due to standardized endotoxin being pre-deposited onto microplates that are stable and stored at room temperature.
- **Validation and metrology:** System implementation and cGMP release are greatly improved with newer technologies that can be validated in days. It is important to scrutinize validation processes and ensure timely validation can be achieved with a new platform. Additionally, considerations should be made based on how many final products can be validated and how complex maintenance will be for a given platform. For a deeper dive into validation of endotoxin testing platforms, see Chapter 7.
- **QC Lab:** The central hub for any endotoxin testing program is the QC lab. Here is where hands-on experience with a platform occurs, as well as the opportunity to increase throughput, decrease assay setup time, and improve other operational metrics such as retest rates. Considerations for new technology implementation include QC analyst training, hands-on time, software management, and analytical method comparability studies. Technologies that make day-to-day QC testing easier and faster – while not requiring difficult training or method transfer processes – are the most beneficial. Software can also play a large role in terms of usability, adoption, and speed in the QC lab.



- **QA and Regulatory:** When it comes to ensuring that products meet quality standards (including internal, industry, and safety standards) and comply with all government regulations, QA and Regulatory professionals in pharmaceutical manufacturing are committed to delivering safe, high-quality medicines to patients in the most efficient manner possible. However, in order to not sacrifice quality, sometimes speed and efficiency are compromised. When assessing new technologies, there are clear “must haves” in the categories of data integrity, compliance, security, and ability to withstand the pressures of an audit. Once those criteria are met, additional value is realized through fast and easy processes for data review, sign off, and batch release.
- **IT and IS:** Enterprise software solutions that maximize flexibility while still ensuring compliance offer the capabilities needed for today’s data security and scalability needs. IT and IS teams supporting drug manufacturing, testing, and release for distribution to the market can do so more efficiently with technologies that offer customization of permissions; ease of data security, access, and management; and simplicity of integration achieved through a single software program. Data integrity and 21 CFR Part 11 requirements must be met with complete confidence.

SIMPLIFIED ENDOTOXIN TESTING FROM INVENTORY TO BATCH RELEASE

Sievers Eclipse* BET Platform

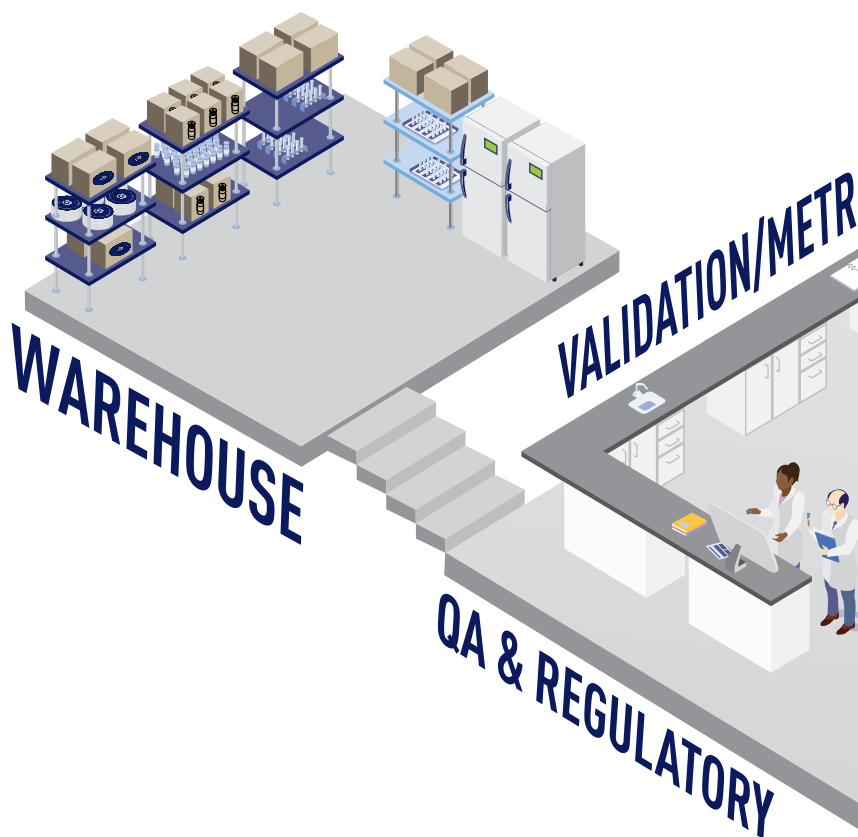


The **Sievers Eclipse Bacterial Endotoxins Testing (BET) Platform** uses microfluidic automation and the latest in compliant software to simplify each step of your endotoxin testing program, from supply chain and storage to validation, routine testing, and sign off.

1

**REDUCE costs and
minimize storage with:**

- Room temperature storage of microplates
- 10x reduction in LAL cold storage
- No RSE or CSE needed — standard endotoxin is pre-deposited on the Eclipse microplate



4

SIMPLIFY product release with:

- Streamlined batch testing, data review, and release
- Remote data review and sample sign off
- Full assay-specific audit trail review

2 IMPROVE system implementation and cGMP release with:

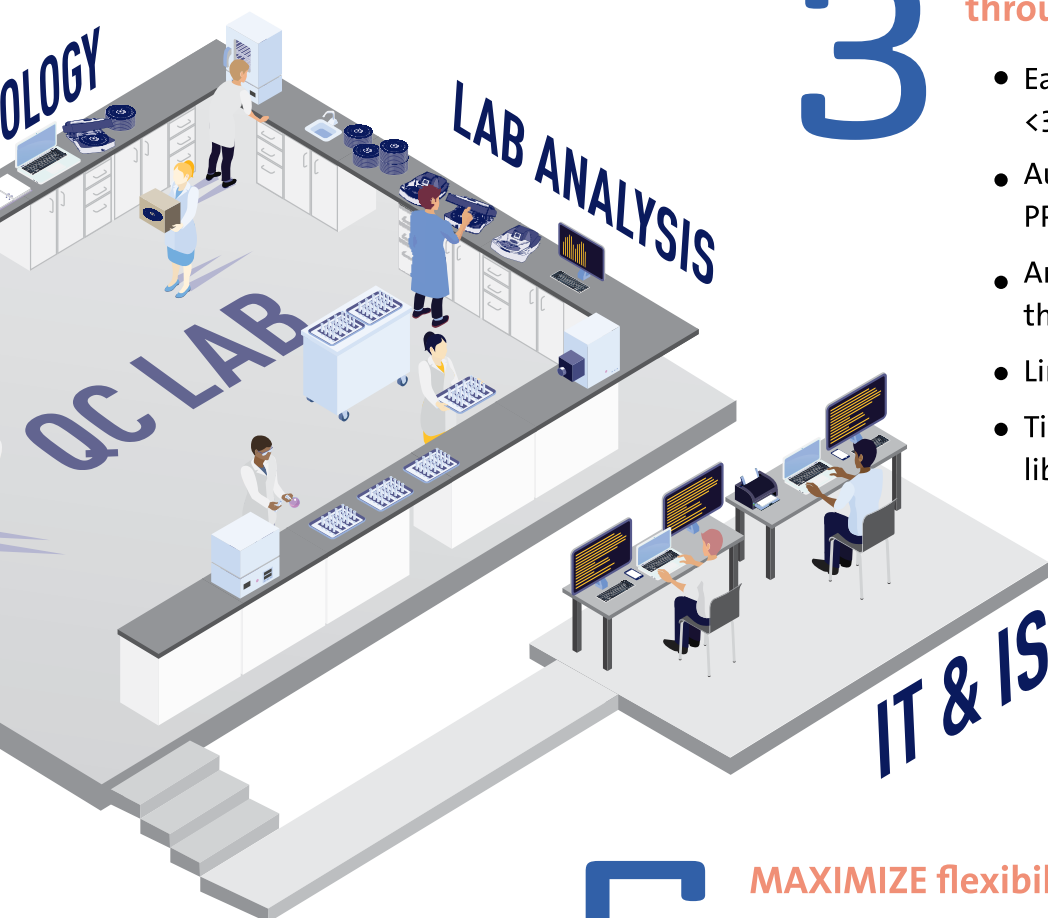
- System validation in days
- Ability to validate 7 final products per Eclipse microplate
- Minimal maintenance of benchtop analyzer — no robotics

3 STREAMLINE and increase throughout with:

- Easy assay setup in 9 minutes, <30 pipetting steps
- Automation of standard curve, PPCs, and mixing
- Analyst training in a fraction of the time
- Limited opportunity for error
- Time-saving templates and libraries

5 MAXIMIZE flexibility and ensure compliance with:

- Enterprise software solution
- Customization of permissions
- Data integrity and ALCOA+
- 21 CFR Part 11



Preparing the QC Lab for a New System

After reviewing various areas of a facility that can benefit from BET automation, it's time to take a deeper dive into key steps needed to prepare for a new system. As discussed in Chapter 2, the goal is to choose or create a route which avoids major roadblocks. Often, this starts with method suitability testing. Confirming that a new testing platform will generate suitable and comparable results for your samples is an important first step that can be completed in-house in the QC lab, or by the platform supplier. QC labs that are resource constrained or don't prefer to do this type of testing in-house should partner with suppliers that provide support throughout the transition, including for method suitability testing.

Spotlight on Method Suitability and Comparability Testing:

Confirming that a new platform will provide suitable and comparable results for your samples is a necessary step along the path to automated BET. However; don't let the time commitment for this testing be the roadblock to moving forward. Partnering with your BET platform supplier to get this testing completed quickly is a great option to save QC labs time and ensure confidence in a new system.



Validation of Endotoxin Testing Platforms

Once method suitability studies are completed, labs are ready to move forward with implementation of a new platform. Validation is often the step that can slow progress if platforms are complex or suppliers do not provide support for the validation process. But it doesn't have to be.

There are several guidelines, principles, and documentation that must be completed and followed precisely to ensure a successful validation and smooth progress toward the ultimate destination of automated BET. Instrument qualification is a frequently cited deviation in regulatory audits, making the potential of warning letters even more intimidating while going through the validation process. If the deviation is critical enough, it could shut down production, turning into a costly and timely error.

What does validation require today?

- Validation of a BET platform can involve following good automated manufacturing practice (GAMP) principles, guidance from USP general chapter <1058> "Analytical Instrumentation Qualification," as well as documents for design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ).
- To ensure the entire platform is properly validated, GAMP principles, together with ALCOA+ and 21 CFR Part 11 guidelines, are used to supplement USP <1058> and help address any gaps related to software validation. One of the most common reasons for noncompliance stems from unclear or improper interpretation of guidelines and terminology.
- Despite using current resources and guidelines, a lab that wants to implement a new or alternative analytical method entirely may also need to complete additional testing outlined in USP <1225> "Validation of Compendial Methods" or ICH Q2(R1) "Validation of Analytical Procedures."
- Many vendors provide the documentation to complete IQ, OQ, and PQ testing of new instrumentation and software. This is a welcome relief to some QC labs, while others see it as yet another hurdle, should the vendor-provided documentation not meet the requirements outlined in their organization's Quality Management System (QMS). The sheer scope of work required to complete a full IQ/OQ/PQ, especially related to more complex instrumentation, can result in some users delaying the implementation of the new platform until there is enough time to complete it without interruption.
- Once validation of the platform has been completed, standard operating procedures (SOPs) will likely need to be created or updated. This additional step is required before the platform can be integrated into the lab for routine use.

What are some validation challenges when moving to automated BET?

Different endotoxin testing platforms can present unique challenges when it comes to validation. These challenges should be considered in advance in order to avoid roadblocks. The last thing a lab wants is to have a new BET platform sitting idle because the time and complexity of validation overpower the available resources to move the platform into routine use. Here are areas of caution to watch for related to validation:

- When it comes to robotics, QC labs may struggle with the time needed to validate the complex actions of these technologies, or they may need a dedicated, highly skilled engineer to spend a significant amount of time operating and validating the instrument. Additional test cases may also be required to challenge and verify software scripts and functionality.
- Some platforms with pre-deposited endotoxin and LAL can deviate from the manufacturer's instructions for use (IFU), as the time required to analyze all samples with some robotic platforms would require additional hold-time studies to be incorporated in the validation process. This would require additional resources and add several days to an already time-consuming process.
- Not all platforms can handle difficult substances, making it hard to demonstrate robustness. This could force a lab to utilize multiple platforms in order to meet the demand for all products and water testing.
- Testing beyond the validation process may also be required in some labs, should there be additional questions around the analytical method itself. That testing would follow the guidelines outlined in USP <1225> and ICH Q2(R1) for accuracy, precision, specificity, limit of detection, quantitation limit, linearity, range, and robustness and can add multiple days to the validation process.

What does ideal validation look like?

While validation of a platform can be arduous, there are options available that are streamlined and keep labs functioning at the capacity needed, without disrupting or re-assigning analysts. Simplifying the process allows the QC lab to complete validation in-house or with the help of manufacturer. When you see these signs, it's an indicator you're in the fast lane to easier validation:

- IQ/OQ/PQ documentation that is clear, easy to follow, and comprehensive enables an ideal platform to be fully validated by anyone in the lab within days. Users will have confidence knowing that the instrument and software are fully qualified and validated per the regulations. Such robust qualification ensures that the instrument and software will function as designed, even at full capacity.
- Alternatively, having the validation performed on-site by a qualified and certified manufacturer's representative lets the lab analysts and managers stay focused on other projects with minimal down time. Once validation is complete, a lab manager or validation engineer can simply review the documented results and sign off to support cGMP release of the equipment.
- Streamlining validation and integration of the platform in the lab can be achieved when a manufacturer's representative assists with steps such as configuring the software, training analysts how to use the platform, and pointing out helpful features and shortcuts, such as setting up assay templates, product libraries, validated products and user permissions.

Validation doesn't have to be a daunting task. There are options available to QC labs to speed up and simplify the process, ensuring a clear path to success. With an ideal platform, validation can be performed in just a few days, analysts can be fully trained during that time, and system validation is supported by the vendor's fully documented results for all seven guidelines outlined in USP <1225> and ICH Q2(R1).



Training on a New Platform

With a new system in the lab, training is an opportunity to accelerate the progress toward the goal of automated testing. A strong partnership with your platform vendor enables this to be quick and seamless – even performed in tandem with validation – to get you moving forward with routine testing. While it is imperative to train and qualify all users on a new system, ideally this process can be completed within just a few days, rather than weeks as with alternative platforms. It is also ideal to select platforms where advanced skills and detailed training for technicians aren't required.

Here are ways to keep training short and sweet, but also effective:

- Choose platforms that minimize hands-on steps and tedious protocols
- Make use of training procedures and analyst qualification tools built into software
- Save time by setting up protocol templates and products in libraries



Transitioning and Validating Products

Following validation and training, it's time to transition from your current testing routine to the automated platform. Certain platform vendors can support this process with applications testing to optimize the test method, on-site support of a bridge study, and a bridge study protocol. To formulate a plan, first consider the method or technique currently used for each sample type in your lab. Here are three common examples you may see:

Kinetic Chromogenic → Kinetic Chromogenic on an automated platform

This is the most direct transition because the kinetic chromogenic technique is the same between systems. In this scenario, the biochemistry, or the reaction between samples, standards, and LAL will remain the same due to the consistency of the 1:1 sample to lysate ratio. Once the system is validated, a brief bridge study can be completed to finalize the transition, which consists of side-by-side testing with the existing sample preparation remaining consistent. With an automated platform that is simple and efficient to use, running a bridge study requires little additional time or effort.

Kinetic Turbidimetric → Kinetic Chromogenic

Many QC labs choose to work with the turbidimetric formulation because they want a quantitative endotoxin assay, and the turbidimetric option is more economical for routine testing. Typically, this decision is not due to an incompatibility with the chromogenic method and sample type(s). The simplicity and efficiency gains now achieved with an automated kinetic chromogenic platform provide an economical and sustainable option

for quantitative results. In this scenario, a QC lab should first perform method suitability testing to compare inhibition/enhancement to the turbidimetric technique and demonstrate that endotoxin can be adequately recovered with the chromogenic method. Generally, when switching method or technique it is recommended to perform a three-lot revalidation for final product release testing. Once the dilution required to overcome interference is identified, ideally labs should leverage validation features within software to test the required number of lots when samples are available and obtain clean validation summary reports for each sample that meets the established criteria.

Gel-clot → Kinetic Chromogenic

The gel-clot technique is commonly leveraged for difficult sample matrices and for legacy product final release. However, many labs still use the gel-clot technique for sample types such as water and are eager to implement a simpler quantitative technique. As with the scenario above, users should first perform method suitability testing to confirm that the samples they wish to convert are compatible with the kinetic chromogenic technique by performing inhibition/enhancement testing. For samples that do not require dilution to overcome interfering factors, such as ultrapure water, the process is further simplified. For product samples, this testing can be followed by a three-lot revalidation.

Software's Role in Simplification

In today's QC environment, the role of software in simplification can't be underestimated. Regardless of how impressive any automated technology may be, the lab and the business likely won't realize its true benefits unless the associated software meets the following criteria:

- Adheres to current data integrity and compliance requirements, including 21 CFR Part 11 guidelines and ALCOA+
- Utilizes client-server architecture for easy and remote data review and sign off
- Incorporates customization of permissions
- Integrates seamlessly with the BET automation platform, ideally being purpose built
- Provides time-saving templates and features such as protocols for analyst qualification, product validation, and lysate qualification, plus libraries for easy customization
- Enables full assay-specific audit trail review



BET Automation Checklist

This eBook has described various steps along the path to implementing simplified endotoxin automation. With the ultimate destination in mind and using this eBook as your guide, BET automation is easily within reach. Use the tips and caution signs described to navigate your path, and remember that automated endotoxin testing shouldn't be difficult.

Follow this checklist on your path to simplified endotoxin automation:

- ☑ Consider the ultimate goals of your lab and organization to help choose the right automated technology for you. Consider which pathways you may want to take or avoid.
- ☑ Understand how successful BET automation can positively impact various departments in your facility, not just the QC lab.
- ☑ Ensure method suitability and comparability of a new testing platform.
- ☑ Beware the potential pitfalls of complex system validation and avoid them.
- ☑ Use vendor support as needed for validation and training. Aim to perform validation and training in tandem and within a few days.
- ☑ Rely on the simplicity and efficiency of assay setup on your new automated platform to make steps easier, such as performing bridge studies and method development. Partner with suppliers to further simplify these steps.
- ☑ Remember software's role in simplification, and be sure to take advantage of time-saving templates and libraries. Use enterprise software for easy, remote data management.
- ☑ Never compromise on data integrity or compliance.

Top 5 Tips for Easier Bacterial Endotoxin Testing (BET)



TIP 1

Automate where you can

Automating the liquid handling aspects of endotoxin testing can be achieved using microfluidics or robotics. This includes automating the standard curve preparation and positive product controls for every assay. Reducing the amount of interaction needed during the preparation process automatically reduces the chance for errors and contamination.

TIP 2

Utilize support for platform validation

Taking advantage of support offered by the BET platform manufacturer can streamline the validation process and ensure compliance is achieved. It also removes the stress of finding time and designating people to complete the validation. Utilizing the support that is available means you can start seeing the benefits of your new platform quickly.

TIP 3

Set up libraries and templates in your software

Using libraries in the software to build and save products for testing can make for simplified assay template setup. Assay templates can potentially also be built and saved in another library to streamline routine testing.

TIP 4

Avoid compliance concerns

Ensuring that your BET software and instrument are fully validated can help ease compliance concerns. It is important to verify that data integrity and 21 CFR Part 11 guidelines are addressed. These guidelines are usually outlined in the IQ/OQ/PQ documentation and should be easily identifiable within the software. In terms of meeting all requirements of the harmonized global pharmacopoeia, USP <85>, EP 2.6.14 and JP 4.01, you can avoid compliance concerns by using FDA licensed LAL, qualified consumables and reagent water, and including the following: minimum three-point standard curve in duplicate using standardized endotoxin, samples and PPCs in duplicate, negative controls in duplicate, and analyst and lysate lot qualification in triplicate.

TIP 5

Leverage client-server software

Having client-server software installations located on several computers allows for results to be reviewed and signed off on from home, while traveling, or simply from another location in the facility. This means products can be released more quickly and analysis can continue in the lab as needed for other products.



Microfluidic Automation and the Sievers Eclipse BET Platform - FAQs

Q: What are the benefits of microfluidic automation?

Microfluidic automation is the simplest form of automation available to the market today. It simplifies endotoxin testing in a platform that is extremely easy to set up, use, and maintain. With microfluidic automation comes high throughput, quick assay setup, minimal hands-on time, and easy training. The Sievers Eclipse Bacterial Endotoxins Testing (BET) Platform uses a standard benchtop analyzer in terms of footprint, size, and basic functionality that is paired with the Eclipse microplate to automate assay setup. By leveraging microfluidic liquid handling and the platform's embedded endotoxin standards and positive product controls (PPCs), quality control analysts can easily and quickly begin a fully compliant endotoxin assay in 9 minutes and in as few as 27 pipetting steps, with up to 21 samples.

Another benefit of microfluidic automation relates to pipetting. Pipetting is one of the largest contributors to errors and retests in the endotoxin market, so by reducing the pipetting steps to less than 30, the Eclipse platform mitigates risks and opportunities for errors that lead to costly retests. In addition, the microfluidic liquid handling precisely measures all liquids for the end user. This means that the precision typically required during the physical action of pipetting is eliminated through the precise design of the Eclipse microfluidic microplate. Microfluidic automation enables labs to achieve the high throughput and easy assay setup they want, without having to be concerned about footprint, complex validations, or compliance.

Q: What components make up the Eclipse BET platform?

The Eclipse BET platform consists of three components: (1) An analyzer that is an incubating spectrophotometer, just like other instruments used for kinetic endotoxin testing; (2) A microplate that automates the assay through microfluidic liquid handling, pre-deposited endotoxin standards, and pre-deposited PPCs; (3) Enterprise software that has convenient protocols and libraries, fully customizable permissions, and of course, full compliance with 21 CFR Part 11 and data integrity guidelines.

Q: How does microfluidic automation work?

Within the Eclipse microplate, microfluidic liquid handling facilitates accurate and rapid dispersion of reagents and samples with drastically reduced volumes of sample and LAL. This is achieved using microfluidic channels, metering chambers, and centrifugal force as the microplate spins to control and automate all liquid measurement, flow, and mixing in preparation for analysis. Preloaded standards and PPCs are used to automate standard curves and PPC spikes. In addition, the closed microfluidic system prevents environmental contamination and precisely delivers a 1:1 sample to lysate ratio. With microfluidic automation, compendial endotoxin assays are performed effortlessly and with fewer retests.



Q: Are standard curves automated?

Traditionally, for an endotoxin assay to remain compliant, the end user must construct at least a three-point standard curve in duplicate from a stock vial of standardized endotoxin; must have duplicate negative controls; and must run each sample in duplicate with a PPC, also in duplicate. However, because Eclipse automates these steps with preloaded endotoxin standards spanning up to a five-point standard curve and preloaded PPCs, all the end user has to do is load Water for BET and samples onto the plate with no additional prep work. The result is the ability to set up the assay in 9 minutes, compared to upwards of 60 minutes that other platforms require. With the Eclipse platform, lab technicians are thrilled with how simple and quick assay setup is!

Q: Is it compliant?

Yes. The Eclipse platform uses commercially available, FDA licensed LAL and meets all requirements of the harmonized global pharmacopoeia, USP <85>, EP 2.6.14 and JP 4.01. Regarding data management and integrity, Eclipse software was designed with ALCOA+ principles at the forefront to provide a highly customizable enterprise solution with 21 CFR Part 11 and data integrity compliance features. To summarize, the Eclipse platform includes:

- Minimum three-point standard curve in duplicate using standardized endotoxin
- Samples and PPCs in duplicate
- Negative controls in duplicate
- Analyst and lysate lot qualification in triplicate
- Use of FDA licensed LAL
- Compliance with 21 CFR Part 11 and data integrity guidelines

Q: How much LAL reagent is needed?

With just 1 mL LAL reagent, 21 samples can be run on the Eclipse platform. By decreasing horseshoe crab (HSC) lysate use by up to 90%, the Eclipse reduces the demand on this valuable natural resource and delivers a fully compliant BET assay that the global HSC population can sustain.

Q: Is training difficult?

Since so much of the difficult assay setup is eliminated with the Eclipse, training and analyst certification are extremely straightforward. Once the system is fully validated, an analyst can be trained and certified within one day using the template in the software. This function conveniently allows a lab manager to keep track of who is qualified, unqualified, or due for requalification.

Q: Are there unique considerations around method transfer and validation?

Regardless of the current state of endotoxin testing, transitioning to an automated and efficient platform is extremely straightforward with the Eclipse platform – method transfer, validation, and all. Veolia supports customers with expert guidance for implementation of the Eclipse, including method suitability testing, product validation, and system validation. These necessary steps can take place on site or in Veolia's lab in Boulder, Colorado.

In addition, the Eclipse platform inherently simplifies training programs, analyst and lysate qualifications, system validation, and product validation. A full IQ/OQ/PQ document is available, as are services provided by Veolia to further simplify the implementation of this innovative endotoxin solution.

Q: How are data review and sign off handled with Eclipse?

In today's environment, it is imperative that the data review process allows for security and efficiency. Quality control labs want to readily review and sign off on data and batch release information – and always in a secure manner – in order to release product or in-process materials to continue their manufacturing process. Therefore, an enterprise software solution like the Eclipse that offers secure access from any location is extremely valuable to life science businesses with multiple sites or remote workers.

The Eclipse software has helpful functionality for data review, including the ability to set permissions for each user. If a reviewer needs to differentiate between final product or in-process and raw materials, or perhaps water testing, they're able to do that within the software. Reports can also be viewed for specific samples individually if necessary. All reports are securely tracked within system and assay-specific audit trails per compliance requirements.

As an enterprise solution, the Eclipse software allows for simple, remote access for data review and electronic signature that makes it convenient and efficient for the quality control lab to use. From the analyst to the quality manager and quality assurance professionals, all software users benefit from the secure, flexible options for reviewing and releasing their samples.

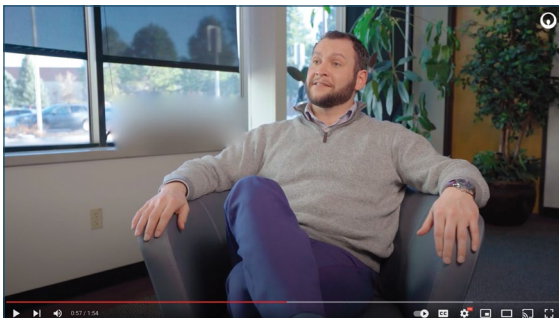
Additional Resources



Sievers Eclipse* BET Platform
Easy Setup



Easy Assay Setup With The Sievers Eclipse BET Platform



Data Integrity & Compliance With
The Sievers Eclipse BET Platform



Innovation & Sustainability With
The Sievers Eclipse BET Platform

To learn more about simplified BET automation with the Sievers Eclipse, visit www.watertechnologies.com/eclipse