Pharma&Biotech

Lonza

Endotoxin Testing

A Comprehensive Training Course

Endotoxin Testing Methodologies and Regulatory Requirements

Course Overview

Lonza's Endotoxin Detection Training Courses are structured around a well integrated combination of lectures dealing with test methodologies and regulatory requirements coupled with practical demonstrations and hands on practical exercises where delegates gain experience with a variety of Endotoxin detection test methods, understanding of assay characteristics and approaches to problem solving.

The training courses are designed on a daily modular basis so that delegates may select the level and content relevant to their learning needs.

In each of the courses, Lonza fosters an open and informal environment where questions and discussion are actively encouraged. The number of delegates for each day course is deliberately restricted to ensure a positive interactive learning environment. Each delegate will have a chance to state their objectives at the beginning of the course and these will be reviewed at the end of the day. A signed training certificate is presented to each delegate upon successful completion of each day course.

As part of our continuing commitment to our customers, Lonza can also offer custom designed Endotoxin Detection Training Courses on site at your company (for a minimum of 4 delegates). The custom courses can include some or all of the standard course content and can include practical demonstration and hands on problem solving sessions.

Introduction to Endotoxin Testing Training

– This course is designed to provide new users with the basic tools to conduct Endotoxin testing within the laboratory and includes practical demonstrations of a variety of detection methods.

Advanced Endotoxin Testing Training

– We know how important reliable product quality is for your day-to-day research. In this course, we therefore use the highest quality standards in the industry so you can rely on the performance of our products, now and in the future.

Practical Endotoxin Testing

– This course is designed for delegates who wish to gain direct practical experience to develop or improve their skills with Endotoxin assays. This course is a full day of practical training including product validation and troubleshooting using the delegate's chosen detection method.

Introduction to Endotoxin Testing Training Course

History and Overview

An initial overview is given of the history of fever and injectable drugs, including the basic research that established the structure and function of the Endotoxin molecule. The discovery and development of the rabbit pyrogen test and LAL Endotoxin detection methods are also discussed in this section.

Methods

All the current Endotoxin detection methods are covered in detail including the most recent development and the probable future of Endotoxin testing, an assay based on recombinant technology and no longer reliant on bleeding the horseshoe crab as a source of raw material. Background, advantages and disadvantages and practical application of each test method are examined.

Practical Demonstration

Practical demonstrations of the following methods using Lonza products:

- Gel Clot using PYROGENT™ Ultra Liquid Standard Gel Clot Kit for use in semi-quantitative gel clot testing
- Kinetic Turbidimetric or Chromogenic (tailored to the delegates' particular interests) using PYROGENT™-5000 or Kinetic-QCL™ Complete Reagent Kits and WinKQCL™ Endotoxin Detection Software for use with plate readers
- Recombinant Factor C using PyroGene™ Endotoxin Detection Method using fluorescent technology and WinKQCL™ Endotoxin Detection Software

The practical demonstration session is intended to give delegates an overview of the methods and an insight into the practicalities of running a test. Time will be allowed for questions. For hands on practical training, please see our Practical Endotoxin Testing Training Session.

Understanding the Data Report

As software systems become more sophisticated both in terms of functionality and to meet regulatory requirements, the information presented in assay reports becomes more complex. It is important that there is a good understanding of how the information provided on the assay report is generated and how to interpret unexpected results. This session will take delegates step by step through a WinKQCL™ Assay Report explaining the significance of each piece of information and how it can be used to control Endotoxin testing in the laboratory.

Endotoxin Testing and Regulatory Requirements

Regulatory requirements are a constantly changing field and our course material is updated to reflect the most recently issued documentation and existing or draft requirements or initiatives from the main regulatory bodies.

This session provides delegates with vital information on:

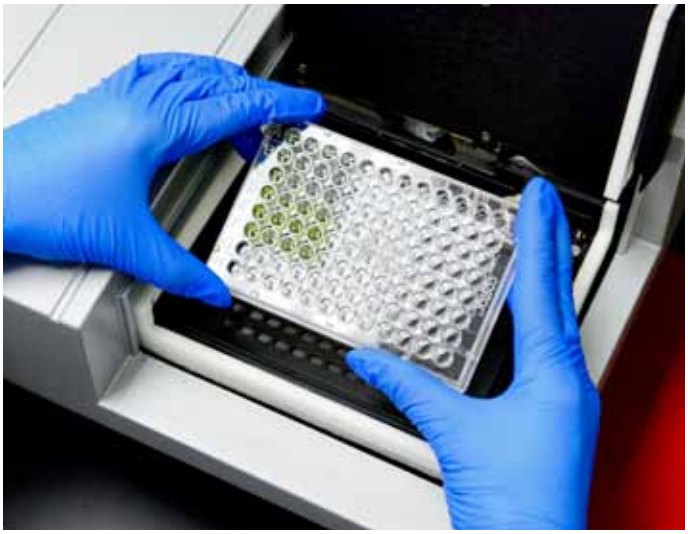
- Who are the regulatory authorities, what documents are relevant, what are the requirements, and what guidance they provide
- How to comply with the requirements for product independent and product dependant validation
- Calculating MVD and using dilution to overcome assay interference
- Practicalities of routine product release testing
- Medical device testing

Common Assay Issues

Validation of the laboratory, equipment, technician, reagents, and products for Endotoxin test methods should mean that a robust method is employed. Due to the physical characteristics of Bacterial Endotoxin and the biological origin of most reagents currently available, variability within the test is inevitable. Common issues such as variable PPC recovery, Endotoxin and %CV results as well as blank reactions are discussed in this section with hints and tips for avoidance.

Advanced Endotoxin Testing Training Course

The Advanced Endotoxin Testing Training Course is intended to give new and experienced users a deeper insight into Endotoxin testing. In order for delegates to gain the most from the advanced day course, we would recommend that delegates have either previously attended an Endotoxin Detection Training Course, have attended our beginner's day course or have been conducting Endotoxin testing in their laboratory for some time.



Water Testing

Water can be a most vital component in the manufacture of a therapeutic drug or medical device to make, maintain and validate. The importance of adequate water testing routines and the regulatory documents impacting this are discussed including validation and revalidation protocols. Each water system will be designed specific to the manufacturing site and final products to be produced. However most will have similar basic components. It is important to understand where Endotoxin contamination can occur, which water samples to take and where from, which containers to use and the sampling routine procedures to ensure you have good monitoring of the water quality. Trending of water testing results is critical to show good process control.

The Mathematics of Kinetic LAL Testing and Assay Variability

This section of the advanced training course is included in response to delegate requests. The Bacterial Endotoxin test is a test of bioactivity and is not a clearly defined chemical test; as such, variability within the test is inevitably higher than for a simple ELISA or protein assay. Understanding the causes of variability and being able to differentiate between inherent and user introduced variability is an important part of assay control in the QC laboratory.

This section explains:

- How limits were set for PPC recovery
- How to choose which PPC concentration to use
- How log-log transformation of the standard curve affects variation
- How the sensitivity range of kinetic assays were determined
- The effect on variance as the sample Endotoxin value increases
- Standard curve regression methods (linear/polynomial)

Raw Material Testing

Whilst there is no official regulatory requirement to test raw material for Bacterial Endotoxin, the regulatory focus on process control testing is certainly increasing. Auditors now expect raw material to be tested prior to manufacturing of final product and the results become part of final product batch records. Adequate testing of raw material helps to build quality into a final product and can prevent costly mistakes such as adulteration of final product by a contaminated raw material. It is often difficult to assign Endotoxin limits or sampling protocols for raw material. This section provides guidance and suggestions on how to set appropriate limits.

Overcoming Assay Interference

The Introduction to Endotoxin Testing Training Course covers MVD calculations and the use of dilution to overcome assay interference. However, there are some products whose characteristics mean simple dilution may not overcome interference. There are several alternative sample pretreatment methods which can be considered and are discussed in this section.

Depyrogenation

What happens if your product, water or raw material is contaminated with Bacterial Endotoxin? For established products on the market, the manufacturing process should be robust enough to limit the chance of gross contamination, but when you are designing a new production process, Endotoxin removal steps should be considered. Some products such as those which are made in bacterial cell systems will by their very nature contain high Bacterial Endotoxin levels. This section discusses Endotoxin inactivation and removal methods that can be employed.

OOS/OOT

What happens when you get an unexpected result? This could be a poor PPC recovery, a higher than expected Endotoxin load, a poor %CV, an issue with the standard curve parameters, or a simple blank reaction/contamination. This section will discuss why it is important to conduct well planned OOS/OOT investigations, when they should be conducted and with whom to discuss the results. The section also contains a case study as a helpful guide to how a good OOS/OOT investigation can prevent future assay or product batch failures.

Clinical Implications of Endotoxin

This section looks more closely at the structure and function of Bacterial Endotoxin in the clinical situation. Delegates will gain a good understanding of the development of sepsis and septic shock from the clinical to the molecular level.

Glucans

The LAL assay methods are subject to false positive reaction in the presence of LAL-reactive material, usually glucan-like in character. This section explains what glucans are, where they are found and what to do if you suspect your Endotoxin positive results may in fact be glucan contamination. The clinical relevance of glucan contamination of your product is also discussed.

The Making of LAL

The horseshoe crab is a living fossil. Discover how the crab is sourced, bled, tagged, and returned to its natural environment. Let us take you through the production process from raw lysate through QC testing and the vialing of the final product. This section will also describe how the new recombinant Endotoxin detection reagent system is made. This section helps provide insight into differences in lysate formulations and how they impact product testing.

Advanced Endotoxin Testing Training Course

The Practical Training Day is designed to provide training on a flexible basis for a small group of delegates to learn more about the practical application of the Endotoxin detection method of their choice.

Delegates will learn a variety of useful practical skills that will help develop and improve their ability to run Endotoxin assays and obtain accurate and reproducible results. The course is deliberately informal to aid open discussion and learning. A work book is provided to guide the delegate through the assay procedures and work exercises and a signed training certificate will be given upon successful completion of the course.

Particular attention is paid to helping delegates become comfortable with the assay, how to deal with difficult products and how to troubleshoot in the event of unexpected results.

In order to ensure individual attention, each practical course is limited to a maximum of 6 delegates. If you are interested in our practical course, but the scheduled courses are full, please do log your interest with your local Product Specialist who will contact you when new dates are being scheduled.

The course will include the following sections:

Supporting Theory for the Practical Course

- Which method to choose?
- Validation from a practical stand point including an outline of equipment IOPQ
- Collection and review of information needed before starting assays for reagent and product validation

Reagent Validation

- The Initial Qualification Test (also known as Confirmation of Lysate Sensitivity Test or Confirmation of Linearity Test). This test is often used to train and validate technicians prior to conducting routine or validation testing. A brief outline of the test and the requirements is presented prior to the practical work and each delegate is given the chance to conduct this assay.

Product Screening (also known as Inhibition Enhancement Testing)

- Each delegate will be given at least one product, for which they will calculate the Endotoxin limit, MVD and PFC for the product and set-up and run a screening assay to determine suitable dilution(s) where the product least interferes with the assay. The assay results for each delegate's product will be discussed as well as the practical use of product pretreatment methods used where simple dilution is not adequate to overcome assay interference.

Product Validation

- Validation of the product given is conducted by testing 3 samples from 3 different lots of the product at the dilution determined in the product screening assay. This assay shows assay reproducibility.

Try Another Method

- Time permitting, the delegates are given the option to try another Endotoxin detection method testing a pre-validated product.

Speakers

The Lonza Endotoxin Detection Training Course was designed by the following experts:

Alan Baines

Alan Baines is currently the Head of Strategic Projects for Lonza BioScience. An Honours graduate in Pharmacology from Leeds University, he has over 35 years of experience in Endotoxin testing since the LAL test was first introduced to the UK in the mid 1970's. Alan founded Lonza's UK operation in 1992, was appointed European Director for the whole of the European life science business in 1998 and subsequently became the director for the European Endotoxin Detection Strategic Business Unit, which was formed in 2001. In early 2004, Alan assumed overall global responsibilities for the endotoxin detection business unit before being promoted in 2012 to the role of Head of Strategic Projects for Lonza Bioscience. Alan presents regularly at PDA and PMF forums and training courses in both the USA and Europe and is the author of the chapter „Routine Testing" in „The Bacterial Endotoxins Test; A Practical Guide", published by the PDA in 2011.

David Guy

David Guy is the European Director of Sales for Testing Solutions within Lonza Bioscience. Based in the UK, David has over 20 years' experience in Endotoxin testing starting as a Product Specialist 1992 and being heavily involved with the introduction of kinetic endotoxin testing systems to the UK between 1992 and 1998.
David managed the UK business between 1998 and 2001, progressing to become the LAL Business Development Manager for Europe until 2004 when he was promoted to European Director for Lonza's newly formed Endotoxin Detection business unit. During this time, David has given many training courses for endotoxin testing in the UK and across Europe. An expert in the computing and instrumentation side of endotoxin testing systems, David has been involved in the developmental processes for robotic testing platforms, a number of versions of Lonza's WinKQCL™ software.
Prior to working within endotoxin testing, David was involved in the sales and support of cell culture bioreactor systems and flow cytometry systems.

Simon Jackson

Professor Simon Jackson, BSc PhD, is Professor of Environment and Human Health and Director of the Centre for Research in Translational Biomedicine at Plymouth University. Simon has over twenty years experience in Endotoxin research and in particular the innate immune response to Endotoxin and its role in sepsis. He has produced over 190 peer-reviewed publications, book chapters and numerous presentations at international scientific and medical research conferences. Simon is also an advisor and reviewer for research councils, research charities, science and medical journals, acts as a consultant to several organisations and is on the editorial board of the journal Innate Immunity. Throughout a distinguished research career, Simon has been closely involved with the measurement of Endotoxin in both basic research and applied clinical and environmental settings and has been responsible for setting up and monitoring systems for plasma Endotoxin in a number of clinical trials of anti-endotoxin and anti-sepsis therapies. Previous posts include Director of Biomedical Research at the University of the West of England, Bristol, Research Director, Medical Microbiology, Cardiff University School of Medicine and visiting scientist, Dartmouth Medical School, New Hampshire, USA.

Ruth Noé

Ruth Noé (BSc (Hons) Medical Microbiology, Leeds University 1994), is the UK & Ireland Sales Manager for Testing Solutions within Lonza Bioscience Products division of Lonza. Ruth has been with Lonza since 2001, first as a Product Specialist (north UK) and since 2004 as Sales Manager. Ruth's role includes sales, support, technical advising, troubleshooting, validation, equipment installation and maintenance, training, service document control, safety representation and line management. Previous to her employ with Lonza, Ruth was a Biotechnology Business Development Manager for 3 years with a CRO working with customers to design their preclinical product biosafety testing protocols (including adventitious virus, bacterial and endotoxin detection test methods). Ruth has also spent time in research laboratories testing blood and bone marrow samples using various molecular biological techniques and in routine virology laboratories in the public health sector.

www.lonza.com
www.lonza.com/lalseminar

Contact Information

North America
Customer Service: 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International
Contact your local Lonza distributor
Customer Service: +1 301 898 7025
Fax: +1 301 845 8291
scientific.support@lonza.com

International Offices

Australia	+ 61 3 9550 0883
Austria	0800 201 538 (toll free)
Belgium	+ 32 87 321 611
Brazil	+ 55 11 2069 8800
Denmark	808 83 159 (toll free)
France	0800 91 19 81 (toll free)
Germany	0800 182 52 87 (toll free)
India	+91 40 4123 4000
Ireland	1 800 654 253 (toll free)
Italy	0800 789 888 (toll free)
Japan	+ 81 3 6264 0660
Norway	800 16 557 (toll free)
Poland	+ 48 781 120 300
Singapore	+ 65 6521 4379
Spain	900 963 298 (toll free)
Sweden	020 790 220 (toll free)
Switzerland	0800 83 86 20 (toll free)
The Netherlands	0800 022 4525 (toll free)
United Kingdom	0808 234 97 88 (toll free)

Lonza Walkersville, Inc. – Walkersville, MD 21793

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