

Shaping the Present and Future of Endotoxin Testing

Trends discussed during Lonza's first Global Endotoxin Testing Summit

Introduction

The Lonza Bioscience Solutions team recently hosted the first Global Endotoxin Testing Summit in Annapolis, MD, USA and Pickering Beach, DE, USA. The event in June 2015 brought the endotoxin testing community together, including pharmaceutical manufacturers, regulatory bodies, vendors and conservation groups, during a very interesting and dynamic time for the industry.

The summit included discussions on the industry's hottest topics. These included the evolution of endotoxin detection methodologies, horseshoe crab conservation, the issue of low endotoxin recovery (LER), an overview of the validation procedure required when using an alternative endotoxin testing method, such as Lonza's PyroGene™ recombinant Factor C (rFC) assay, as well as the use of automation in quality control (QC) laboratories. The latest thinking and perspectives on these key issues are discussed below.

The Need to Safeguard LAL and TAL Supply

The Limulus Amebocyte Lysate (LAL) assay is the current method of choice for testing parenteral pharmaceuticals for the presence of bacterial endotoxins (regulators also permit the Tachypleus Amebocyte Lysate (TAL) assay, which is only used in Asia). There is a growing need for LAL/TAL testing due to a growth in the demand for medicinal products (including biologics). This is because, as more countries become 'economically developed', healthcare needs will inevitably increase. In turn, this will likely lead to a rise in the production of parenterally-administered pharmaceuticals, all of which will need to undergo endotoxin testing. If all of these tests are carried out using the LAL/TAL assay then this could lead to a supply shortage, especially if lysate resources are not managed properly.

The supply of the LAL and TAL tests is dependent on the horseshoe crab (*Limulus polyphemus* for the LAL assay or *Tachypleus gigas* and *Tachypleus tridentatus* for the TAL assay). With evolutionary ancestors dating back to the Paleozoic era, 540 to 248 million years ago, these crabs are thought to predate humans and dinosaurs. LAL/TAL is obtained from the blue blood of the horseshoe crab, which reacts to the presence of bacterial endotoxins and has been used for endotoxin testing since its commercialization in the 1970s. Should anything happen to the population of these crabs, the world's supply of LAL/TAL-based tests would be in jeopardy.



Figure 1. Horseshoe crab (*Limulus polyphemus*) on Pickering Beach.

In order to prevent crab populations from becoming endangered, careful management and regulation is required. In the US, regulations have been put in place, leading to the safeguard of the North American species of horseshoe crabs, *L. polyphemus*. This includes, amongst others, a state-by-state fishing quota established by the Atlantic States Marine Fisheries Commission. Lonza also supports related horseshoe crab and conservation efforts, such as the Just Flip 'em® and Backyard Stewardship™ community horseshoe crab sanctuary program run by the Ecological Research and Development Group, Inc. (ERDG), whose mission is to conserve the world's four horseshoe crab species. Summit delegates had the opportunity to join with ERDG's Just flip 'em!® program

by helping to flip and save stranded horseshoe crabs during an excursion to Pickering Beach, Delaware, one of ERDG's community sanctuaries and a major breeding horseshoe crab site (Figure 2).



Figure 2. Lonza's Global Endotoxin Summit delegates taking part in the Just Flip 'em' program to help save stranded horseshoe crabs

However, unlike in the US, a lack of implemented regulations in Asia has already led to a substantial decline in two related species (*Tachypleus gigas* and *Tachypleus tridentatus*), as highlighted in the presentation given by Glenn Gauvry, Founder and Director of the ERDG and recently published in "Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management" (Springer Publishing).

The most effective means of preserving the horseshoe crab and lysate supply requires a partnership between the pharmaceutical industry, regulatory bodies, vendors, end-users and conservationists. Those involved in endotoxin testing can help, by developing alternative test methods to safeguard the supply. As Gauvry explained, "Working with industry in partnership, we can help support conservation efforts. Companies like Lonza are easy to work with because they are looking for ways to promote the use of alternative methods and sustainable practices."

Alternative (and Not so Alternative) Testing Methods

The need for LAL/TAL alternatives goes beyond just avoiding using animals. In addition to the fact that LAL/TAL is reliant on a potentially finite resource that is increasingly at risk as the need for supply grows, there are several other benefits of using an alternative compared to the natural LAL/TAL assay. These include improved lot-to-lot consistency, enhanced endotoxin specificity, statistically robust spike recovery and ease-of-use, all while meeting the criteria for a suitable sensitivity range (0.005 – 5 EU/ml).

Lonza's rFC, PyroGene™ Assay is a powerful alternative to the LAL/TAL assay. The Food and Drug Administration (FDA) and European Pharmacopoeia (EP) both acknowledge the rFC method as an alternative

means of endotoxin detection. The FDA has listed this in their 2012 Q&A document and outline the validation procedure for this alternative method in USP <1225>. The EP Chapter 5.1.10 will come into effect in July 2016. However, those working in the pharmacopoeias are keen to discuss how rFC-based tests are defined.

As Ingo Spreitzer, Deputy Head of the Microbial Safety Department of the Paul-Ehrlich-Institute (PEI) and European Directorate for the Quality of Medicines and Healthcare (EDQM), whose group published the new EP Chapter 5.1.10, Bacterial Endotoxins Ph. Eur. Policy for Substances for Pharmaceutical Use, expressed in an interview shortly after the Summit: "I hope to see increased usage of rFC in the field of endotoxin testing. This shouldn't be too challenging in my opinion, as I don't see a significant difference between the LAL assay prepared from crab and industry prepared rFC – both depend on Factor C. We have prepared pharmacopeial guidelines listing rFC as an alternative assay, but in the future it would be beneficial if the pharmacopoeia were to make a stronger statement about this issue, especially if horseshoe crab numbers were to go down and put greater pressure on the supply chain."

This is an important issue; the fact that rFC is defined by the regulatory bodies as an alternative test might be holding some back from adopting it because alternative tests require an additional validation procedure to be carried out prior to use.

Compared to compendial methods, the overall effort to validate an alternative does require additional time to implement. However, the steps needed to validate an alternative method could be accomplished in as little as 1–3 days. This can be achieved with ease if following a simple, well-structured protocol that will generate sufficient data to satisfy the regulatory requirements for approval, especially when using documentation and protocols that have already been developed by test vendors.

Low Endotoxin Recovery – a Real Problem, but One that Can be Addressed

LER is the masking of known amounts of endotoxin that have been added to undiluted materials and can be attributed to various combinations of excipients used in drug formulation. It is characterized by a time-dependent failure to adequately recover added endotoxin, resulting in a false negative and calling into question the validity of results when testing certain products.

The common consensus is that LER is a real challenge that must be overcome. In the eyes of Dr. Spreitzer, "LER is the most important issue in endotoxin testing today because it is affecting the testing of current products that are already on the market."

Regulatory authorities consider LER a problem, especially if products contain polysorbate and citrate. However, as of yet there is no method that has been accepted or agreed upon to overcome LER.

As Lonza's Regulatory Affairs Manager, Allen Burgenson, explained: "LER is a new type of inhibition. We've seen inhibition before and we solved it. There are a number of methods by which LER can be overcome before you have to go into the chemistry of demasking. The issue of LER is manageable and while I think is an important technical problem, we do not necessarily have a public health problem."

Several mechanisms have been proposed as to why LER occurs and how these might be overcome. Research carried out by Johannes Reich at the University of Regensburg in conjunction with Hyglos GmbH has begun to investigate the aggregation and interaction behavior of lipopolysaccharides (LPS) (endotoxins) and the related activity in LAL-based detection systems. His research is based on the hypothesis that LER is caused by the breakdown of endotoxin aggregates to monomers which are embedded in surfactant micelles, and the assumption that the aggregation status of the lipopolysaccharide is reversible. This would mean that endotoxin demasking should be possible. As such, demasking will involve re-assembly of the endotoxin aggregates by pushing the equilibrium towards the aggregate state by, for example, adjustments in pH, magnesium and calcium, and/or the addition of polyanionic dispersants such as Pyrospense™.

The data presented by Johannes Reich was very compelling, and while some progress is being made towards developing a demasking process, LER remains an area of active investigation within the industry. Further research into this area will ensure that the issue of LER is addressed and overcome once and for all.

The Future of Endotoxin Testing

LER issues still need to be resolved

Regulators, test vendors and pharmaceutical manufacturers are aware of LER and will continue to look for a solution. Alan Baines, Lonza's Head of Strategic Projects, suggested: "When considering new biological license applications, manufacturers should be aware of the potential issues with LER when using polysorbate in combination with citrate or phosphate buffers. Finding the right combination could side-step potential problems. For existing products, it is likely that additional sample treatment steps will be needed to overcome the masking effect, as changes to formulations are usually a much less attractive option."

Kevin Williams, Senior Scientist for Endotoxin Detection at Lonza, thinks new sample treatment may be required, at least for some product types. He said: "At the moment, many people are resisting using the demasking protocol, as they want to maintain the simplicity of the test. I can certainly understand. However, you can't maintain simplicity by denying that complexity exists. So what we hope to do is to inform and educate at least the manufacturers of biologics such as monoclonal antibodies about the importance of addressing LER, as these are life-saving drugs and it's a significant and growing market area."

A lysate shortage is likely and alternatives are needed

There is concern amongst the endotoxin testing community regarding the supply of LAL and TAL. Hence, the need to protect the existing crab populations and identify alternative tests priorities for the future.

Glenn Gauvry, who works to conserve the horseshoe crab populations, is certainly concerned. "A complete collapse in the supply of TAL would be a big problem," he said. "Asian markets are growing and have an increasing number of pharmaceuticals that will need to be tested. In addition the TAL resources are dwindling, so rFC could be an attractive alternative."

This was echoed by Alan Baines. "I think there will be a shortage of TAL in five to ten years and we won't have the supply to meet the demand," he said. "The industry will then be in a position where they need to more readily adopt rFC."

Johannes Reich was even more confident: "In the next ten years, rFC will be the method of choice because it is cheaper, has less variability from lot-to-lot and isn't limited by supply."

According to Allen Burgenson, in order for rFC to become common practice throughout the industry, "We'll need big players to adopt this so that others will follow and this will increase the chance of it being accepted by regulatory bodies."

A move towards improving the automation and efficiency of testing

Automation of the endotoxin testing process could help the industry cope with ever increasing demand. Alan Baines thinks "Automation of the preparation steps will be possible. In fact for large-scale users this has already been successful, but this is expensive for the application and out-of-scale with the need of the assay." He went on to say that, "Testing is moving from the QC laboratory to the manufacturing floor, with the hope that this will allow problems to be detected sooner and prevented at an earlier stage. This has had some limited success so far, but this could become much more prevalent over the next ten years."

Wolfgang Mutter, General Manager of Hyglos GmbH, agreed: "If we have the technology I think we can manage this." However, he is concerned that, if the pharmacopeial guidelines do not progress alongside the technological developments, this could mean that "... rather than using the latest biochemistry, such as rFC, new automation processes will be designed using LAL reagents, even though they could soon become obsolete." Therefore, the pharmacopeias will need to be kept abreast of the changes in endotoxin testing so that they are able to adjust the regulations in a timely manner. This will encourage the most advanced biochemistry to be used when designing the technology and help ensure that the instrumentation in use is more likely to be well-adapted for the future of endotoxin testing.

The pharmacopeial guidelines will need to be adapted

With all this change, regulators will have to act fast to keep up. As Dr. Spreitzer noted, "The methods in pharmacopeia are very good but the technological progress in the pharmaceutical industry is much faster than the progress of the pharmacopeias. This is okay, as the pharmacopeia exist this way to ensure changes will be sustainable and for the good of the consumer and the industry, but new products challenge the given recommendations and will encourage change." He went on to explain that, "New types of products are going to affect the future of endotoxin testing. I predict that we will need to develop a much more detailed and product-specific set of risk assessments in the future." Every stakeholder will need to be involved in this process, in order to drive the industry forward as fast as possible.

Closing Remarks

A collaborative approach to endotoxin testing between regulatory bodies, pharmaceutical manufacturers, reagent vendors and horseshoe crab conservationists will facilitate the further development and adoption of optimal methods. Research is now being directed at overcoming various

problems; LER for example, may be resolved in the near future, preventing it becoming a risk to human health. If the pharmaceutical industry is encouraged to carry out the necessary validation procedures required for alternative testing, it could improve upon current tests and contribute towards preserving horseshoe crab populations. Furthermore, a greater awareness and acceptance of these alternatives will mean instruments can be designed to accommodate these and enable the QC process to become more efficient. This could lead to the widespread adoption of these new methodologies and aid in supporting faster change amongst the pharmacopeias.

Looking Forwards to 2016

One thing seems certain, change is coming, and the industry will need to operate as a team to ensure that patient safety is always at the forefront of future endeavors. Next year Lonza's Global Endotoxin Testing Summit 2016 will help to bring the community together to review the progress made and to continue to forage a roadmap for the future. To be involved, please visit www.lonza.com/endosummit.

Contact Information

North America

Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 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
Belgium	+ 32 87 321 611
Brazil	+ 55 11 2069 8800
France	0800 91 19 81 (toll free)
Germany	0800 182 52 87 (toll free)
India	+91 40 4123 4000
Japan	+ 81 3 6264 0660
Luxemburg	+ 32 87 321 611
Singapore	+ 65 6521 4379
The Netherlands	0800 022 4525 (toll free)
United Kingdom	0808 234 97 88 (toll free)

Lonza Walkersville, Inc. – Walkersville, MD 21793

Unless otherwise noted, all trademarks herein are marks of the Lonza Group Ltd or its affiliates. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group Ltd or its affiliates and the information and recommendations given by Lonza Group Ltd or its affiliates are (i) suitable for intended process or purpose, (ii) in compliance with environmental, health and safety regulations, and (iii) will not infringe any third party's intellectual property rights.

©2015 Lonza. All rights reserved.
CD-RT-PN030 09/15
