Sustainability in Bacterial Endotoxin Testing (BET) – A Holistic Approach to Conservation and Recombinant Technology

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***Veronika** has over 13 of years of experience in endotoxin testing, and currently manages the global technical team at ACC and is based at ACC's US Headquarters in East Falmouth, Massachusetts.*

Veronika is a subject matter expert when it comes to endotoxin testing and often provides expert sessions at global events focused on BET products and processes. Most recently Veronika has been speaking on the topic of recombinant technology as it relates to BET in the industry and abroad.

Veronika is a key contributor to ACC's sustainability initiatives and spokesperson on ACCs related projects, products and services.

Bacterial endotoxins can cause harmful symptoms, including fever and septic shock, if they find their way into a patient's bloodstream in sufficient concentrations. As a result, Bacterial Endotoxin Testing (BET) has become a fundamental safety requirement in the biopharma industry. Manufacturers must show that their finished products do not contain endotoxins exceeding the allowed limits.

The industry standard reagent for BET is Limulus Amebocyte Lysate (LAL), which is extracted from the white blood cells of the Atlantic horseshoe crab (Limulus polyphemus). For the past four decades, LAL reagents have been the only type of reagent approved by the US Food and Drug Administration (FDA) to test for bacterial endotoxins. In recent years, however, a new class of BET reagents have emerged: recombinant reagents.

Recombinant reagents are non-animal-based and produced using recombinant DNA technology – an attractive proposition for manufacturers looking to reduce their environmental footprint. Thanks to the fact that the recombinant reagents are non-animal-based, they may yield more reproducible and repeatable data. But do they perform as well as the industry standard LAL? That question is still being debated by the subject matter experts, though published studies show extremely promising data. As alternative reagents for testing of products per compendia, the recombinant reagent used has to be shown equivalent to LAL for each individual product tested. This presents some significant regulatory burdens currently associated with recombinant reagents.

First and foremost, the FDA does not license recombinant reagents and will not accept their use unless a compendial test has been performed showing that the reagent is equivalent to LAL. Crucially, this must be done by the individual end user in their own lab – a significant drain on resources. In addition, companies may struggle to understand exactly what the regulatory expectations are, especially given that local regulations and regulatory authorities in different jurisdictions have varying expectations of what they would like to see from the end user when validating an alternative reagent. The regulatory requirements for LAL reagents were harmonized over 20 years ago, but this isn't the case for recombinant reagents.

We are hopeful that these requirements will be harmonized in the coming years – and there are several groups working on this – but compendial testing remains a significant hurdle to the more widespread adoption of recombinant reagents as alternatives to traditional LAL reagents.

Making life as easy as possible

Given the substantial regulatory hurdles associated with implementing an LAL alternative, Associates of Cape Cod, Inc. (ACC) have set out to make things as easy as possible for the end user. ACC's PyroSmart NextGen™ recombinant Cascade Reagent (rCR) is the first and only reagent available on the market that mimics the LAL cascade – the reagent's mechanism of action – completely. This rCR is based on the genetic sequence of Limulus polyphemus and reacts with endotoxins in the same way as LAL. It launched in spring 2021 and is now commercially available globally.

The time to result with PyroSmart NextGen™ can be reproducibly achieved for the sensitivity of 0.005 EU/mL in 60 minutes (including preparation and test time), whereas traditional LAL reagents usually take 85 minutes or longer and rFC reagents (first generation recombinant reagents) take around 110 minutes – though this can be cut to 74 minutes by using a plate with predisposed CSEs. Unlike first generation rFC recombinant reagents, converting over to PyroSmart NextGen™ (rCR) does not require any changes to the user's current platform used for photometric LAL-based assays. The end user can use the same instruments and data analysis software as they do for traditional LAL; the only difference is the reagent. This really simplifies the process of demonstrating comparability with LAL. A considerable number of companies have joined ACC's evaluation program, which allows them to try the PyroSmart NextGen™ reagent and find out how suitable it is for testing their products while simultaneously collecting the comparability data required by regulators.

There is a lot of interest in alternatives to horseshoe crab-derived LAL reagents – especially as the industry as a whole has become more environmentally conscious over the past decade or so. But a combination of resources and internal knowhow limitations associated with proving comparability is a major hurdle that many end users simply cannot overcome – despite good intentions.

We are hopeful for greater regulatory harmonization to ease the burden on the end user but, until then, the process of adopting and proving comparability must be as straightforward as possible, and we are available to help with that process. We believe that allowing manufacturers to maintain their existing instrumentation and software platform will give more companies the option of choosing a non-animal-based BET reagent.

Created by and originally published in The Medicine Maker.

Another First!

rCR, Brought To You By The BET Pioneers
Associates of Cape Cod, Inc.

PYROSMART™ NEXT GEN
Recombinant *Limulus* Amebocyte Lysate

A History Of Firsts!

First To Introduce An Animal Free, Recombinant LAL Reagent

First Large Scale IVF Program To Introduce Horseshoe Crabs Into The Wild

First To Establish BET Contract Testing Services

First BET Company Licensed By FDA

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MKT#21-075