Tipping point – what is driving the adoption of rFC for bacterial endotoxin testing?

As industry leaders seek strategies to build supply chain resilience, multi-sourcing is driving the adoption of alternative solutions like rFC.

Author: Kevin Williams, Endotoxin Senior Scientist at bioMérieux

OVER THE LAST 20 years, the worldwide value of traded pharmaceutical goods has grown six-fold, from $113 billion in 2000 to $629 billion in 2019.[1] Yet amid such robust growth comes risk to supply chain security as manufacturers become increasingly dependent on a global network of suppliers. In a recent McKinsey Global Institute survey, nearly 50 percent of respondents cited sole sourcing of inputs as a critical vulnerability – and 25 percent pointed to a lack of visibility of supplier risks.[1]

One of the most common strategies for building resilience is to expand the network of suppliers for critical components or raw materials.[1] As industry leaders seek strategies to build supply chain resilience, multi-sourcing is driving the adoption of alternative solutions, which in some cases puts industry one step ahead of regulators. Such is the case with alternative methods for bacterial endotoxin testing (BET), which involves the development of recombinant reagents like Recombinant Factor C (rFC).

With industry's push to build a more diverse global supply chain, we find ourselves at a tipping point for the widespread adoption of alternative solutions like rFC — driven as much by industry, if not more, than regulators.

Finding common ground for sourcing

Both pharmaceutical companies and regulators have the same goal — ensuring continuous supply of safe drugs and therapies. Global pharmaceutical companies depend upon robust global supply chains to fulfil their endotoxin testing requirements to ensure patient safety. rFC is a safe, sustainable endotoxin testing solution that could mitigate many of the adverse trends impacting global supply chain consistency. However, the lack of compendial inclusion by the United States Pharmacopoeia (USP) continues to be a barrier to widespread implementation.

While this limitation mostly affects pharmaceutical companies in the US, the impact is global. In fact, even European users who enjoy the compendial status of rFC face challenges if they want to export their products outside of the EU because gaining regulatory approval in non-EU countries requires them to validate rFC as an alternative method.

Geopolitical threats to supply

It can be argued that the only vigorous remaining populations of horseshoe crabs are on the Atlantic seaboard (*Limulus* is vulnerable to extinction versus threatened with extinction for Asian horseshoe crabs). This population can only be harvested by a limited number of domestic companies who essentially have a regulated monopoly on converting the blood to a formulation for *Limulus* amebocyte lysate (LAL) testing. Pharmaceutical companies located outside of the US have cause for

concern regarding access to supply in shifting geopolitical environments.

In 2013, a large pharmaceutical manufacturer that was building a plant site in China was concerned about supply-chain reliability; it determined that adopting rFC was the best option to secure its supply of testing reagents for product release to the market and to better serve patients.

By proactively diversifying their sources for endotoxin testing materials by adopting rFC, they reinforced their supply-chain resilience, which has been defined as:

> …the ability of a given supply chain to prepare for and adapt to unexpected events; to quickly adjust to sudden disruptive changes that negatively affect supply chain performance; to continue functioning during a disruption (sometimes referred to as 'robustness'); and to recover quickly to its pre-disruption state or a more desirable state.[2]

Many drug manufacturers choose to adopt rFC to protect their supply chain access to critical reagents required to release their drug products against the risk of localised distribution issues. Others have indicated that they favour sourcing materials made through bioproduction because they are inherently more reliable than naturally-sourced materials in terms of supply.

Global supply chain uniformity

Apart from concerns of merely securing the supply of reagents, the desire to obtain a continuous supply of reagents that are not subject to variance from harvesting and formulating and packaging and shipping are also driving companies to adopt rFC. Recombinant reagents do not require a harvest of animals and are not constrained by

these factors directly. The very techniques used to manufacture biologic drugs such as recombinant insulin are used to produce the rFC protein from genetically modified single-celled organisms.

The biggest advantage of using recombinant proteins that do not rely on a naturally-harvested source is consistency; rFC has been demonstrated to be far less variable in terms of repeating constant standard curve properties related to batch-to-batch testing, as shown in **Figure 1.**[3]

Consider that horseshoe crab-derived reagents are a pool of various bled animals with different sexes, age, physical condition, location and genetic composition, etc. Recombinantly produced reagents are produced repeatedly from the same stock of genetically modified single-celled organisms in a bioreactor.

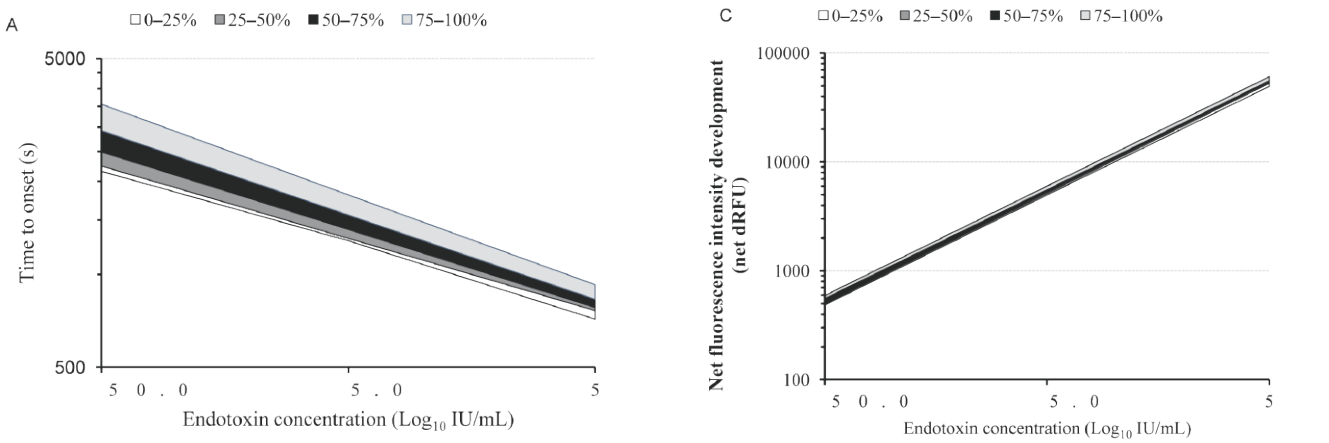
Environmental sustainability goals linked to supply-chain security

Another compelling driver for companies adopting rFC is a sense of environmental responsibility. Given the unpredictable advance of climate change, there is growing evidence of changing temperatures and sea levels affecting natural supply sources. Few would argue that the pressures on both the horseshoe crab and the animals that depend upon them have diminished, in fact extinction pressures continue to build.

There is no question that the recent worldwide expansion of testing for the COVID-19 vaccine and treatments (developmental and marketed) has increased demand for LAL. Some LAL manufacturers are trying to close the gap between supply and increasing demand by expanding their horseshoe crab harvest areas.[4-7]

The recent proposal to loosen restrictions on bait harvesting[8] may also adversely affect horseshoe crab conservation, in that these ongoing efforts can be viewed as in the process of systematically failing. Similarly, shorebirds that depend upon the horseshoe crab egg mass around Delaware Bay may have reached a point of no return on the road to extinction.[9,10]

Figure 1



Standard curve for each assay.[3] **A**) K-QCL and **B**) Endosafe-MCS: time to onset in seconds, on a log10 scale, plotted as a function of endotoxin concentration (IU/ml); **C**) ENDOZYME II GO: net difference in fluorescence intensity development (net dRFU), on a log10 scale, plotted as a function of endotoxin concentration (IU/mL). **B**) Cartridge curve not shown as there is no variability for a factory-performed archive standard curve used repeatedly per batch.

Phased sustainability approach

Changing analytical methods for marketed drugs is expensive and the lack of compendial status for the replacement method can be a real barrier to change. Because of this, many companies are choosing a hybrid sustainability approach – using rFC for non-release endotoxin testing while continuing to release legacy products with LAL.

Water and other raw material-testing constitutes 80-90 percent of all endotoxin test volume. Testing of water and raw material matrices do not require approval from regulators to enact testing that is generally required when an alternative method such as rFC is used for final product release. Thus, rFC can substitute directly for LAL assays in these cases; providing all the holistic advantages discussed with the least amount of pain related to cost of change. Discussions with US Food and Drug Administration (FDA) regulators have considered these types of in-process tests as "inspectional"-based regulation. This means that although validation is important and must be proper and well-documented, it does not require pre-permission to enact.

Building a more resilient future

The pace of regulatory acceptance of new technologies has historically been expedited by an appetite for change in the industry, resulting in increased adoption of these technologies. A tipping point is reached when regulators feel the need to understand a new technology and provide oversight. We have seen examples of this for endotoxin testing specifically in the move from the original gel clot test to kinetic (chromogenic and turbidimetric) testing, resulting in the FDA creating Guidelines (FDA Guidelines/1987/1991) for LAL testing and USP adding compendial status for kinetic testing (USP <85>/2001).

A more holistic, forward leaning view is needed to make urgent upgrades that augment rather than restrain global supply chain resilience. Pharmaceutical manufacturers worldwide should keep an eye on issues affecting both the availability and consistency of LAL batches and work with regulators to find ways to ensure sustainable access to bacterial endotoxin testing reagents. Many regulatory bodies around the world have already granted compendial status to rFC for the sustainability reasons discussed here. More importantly, they have been satisfied by the data generated by the industry that rFC is not only a sustainable solution for endotoxin testing but a safe one. The more industry continues to adopt rFC, the closer we move to global regulatory acceptance and increased supply-chain robustness.

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WASTE REDUCTION IN ENDOTOXIN TESTING PLATFORMS

From traditional to alternate endotoxin testing methods used for product release testing, there is an opportunity to **waste less** reagent and generate **cost savings.**

Waste in Endotoxin Detection Assays: LAL vs rFC

Given that Limulus Amoebocyte Lysate (LAL) is packaged in vials (2.6 or 5.2mL) and reconstituted as needed, there is inevitable waste compared to using recombinant Factor C (rFC). rFC is a liquid reagent that is prepared (combined as enzyme, buffer, and substrate) in a ratio that can be prepared in amounts very close to what is needed (no waste).

	ENDOZYME II GO assay and GOPLATE™ with rFC	ENDOZYME II GO STRIPS with rFC	Cartridge LAL	Traditional LAL
Method	rFC with 96-well microplate pre-filled CSE with required standard curve and positive product control concentrations (PPC). Up to 21 samples per assay.	rFC with 96-well STRIP-based microplate CSE pre-filled with required standard curve and positive product control concentrations (PPC). Up to 21 assays per hour.	Multi-cartridge system uses LAL-cartridge technology to run one sample per cartridge. LAL reagent, chromogenic substrate, and CSE are contained within disposable cartridges. One sample per assay and up to 5 parallel assays.	Traditional LAL assays with 96-well microplates. Standards and samples must be prepared. Involves high-volume pipetting. 21 samples per assay.
Technology	Plate based	STRIPS	Cartridge based	Plate based
Horseshoe Crab Blood	0%	0%		
Turnaround Time (per 21 tests*)	25 MINUTES	25 MINUTES	90 MINUTES	60 MINUTES
Overall Waste**	0%	0%	0%	25-35%
Cost Savings Based on Waste**	25%	25%	N/A	0%
Environmental Impact			ENDANGERED MARINE LIFE	ENDANGERED MARINE LIFE
Supply Chain Security			ENDANGERED MARINE LIFE	ENDANGERED MARINE LIFE

*Marius M, et al. Comparison of Limulus Amoebocyte Lysate and Recombinant Factor C Assays for Endotoxin Detection in Four Human Vaccines with Complex Matrices. DOI: 10.5731/pdajpst.2019.010389. Epub 2020 Mar 16.

**Overall Waste/Cost Savings: Based on reagent use achieved using reconstituted vials (for LAL) versus preparation of volumes needed using liquid rFC reagents.

bioMérieux, Inc. • 100 Rodolphe Street • Durham, NC 27712 • U.S.A. • Tel: (800) 634-7656 • Fax: (800) 657 3053
www.biomerieux-usa.com