Betasciencepress Publishing
Open Access

J Appl Bioanal 9, e23002 (2023).

Journal of Applied Bioanalysis

Expert Opinion Article

Current Challenges and Opportunities in using the LAL Assay for Endotoxin Testing

Timothy Francis

LAL Division, FUJIFILM Wako Chemicals U.S.A. Corporation.
Correspondence: FUJIFILM Wako Chemicals U.S.A. Corporation, 1600 Bellwood Road, Richmond, VA 23237-1326, USA; Phone: +1 8046724655, **E-mail:** timothy.francis@fujifilm.com

First draft submitted: Februari 7, 2023; **Revised:** March 30, 2023; **Accepted for publication:** March 31, 2023.

Keywords: Endotoxin, LAL, Atlantic Horseshoe Crab, Pyrogens.
Editor: Chukwunonso Nwabufo, Univeristy of Toronto, Toronto, Ontario M5S 1A1 Canada.
Funding & Manuscript writing assistance: The author has declared that this work was supported by financial support and that no writing assistance was utilized in the production of this article.
Competing interests: The author is an employee at above mentoined company and has declared that no competing interest exist.

1.0 Introduction

The LAL assay for bacterial endotoxin testing has undergone many changes in recent decades and will continue to do so. From the initial discovery of LAL and gel clot qualitative reagents to the addition of chromogenic substrates for quantitative reagents to enhance the test measurements, the test has developed. However, not all improvements have been realized. Endotoxin testing is now facing some of its greatest challenges. However, challenges lead to further opportunities for growth and development [1].
This discussion will look at a few challenges facing endotoxin testing along with the opportunities that have arisen to meet those challenges.

2.0 Challenges

The first current challenge to endotoxin testing is the supply of materials and conservation of resources. The primary reagent used for Bacterial Endotoxin Testing is Limulus Amoebocyte Lysate (LAL) or Tachypleus Amoebocyte Lysate (TAL). This reagent is derived from a natural source, the hemolymph of either the Atlantic Horseshoe Crab (Limulus polyphemus) or Chinese Horseshoe Crab (Tachypleus tridentatus). Fishing has done much to reduce both populations. The Limulus population has nearly returned to its previous levels. Although exact numbers are hidden due to confidential data, the estimated Atlantic Horseshoe Crab population has steadily increased steadily since 2002 [2]. However, the Tachypleus population continues to decrease. Since the 1990s, the annual harvest for biomedical purposes has dropped from 600,000 to 100,000 in 2015 [3].

In addition to the concerns in population levels, the limitations in ecosystems that allow for the populations of these two species to thrive is also a concern. Limulus only thrives in the shallow waters along the bays and estuaries of the Atlantic coast of the eastern United States as immature extending to the edge of the continental shelf as adults [4]. Tachypleus also lives in coastal and inshore locations. Its range extends from Japan in the north to Indonesia in the south. Both locations are prone to disruption from tropical cyclones. These can extend to temporary interruptions in the supply of the crabs for LAL production to permanent destruction of the crabs' habitat [5]. Two potential challenges present themselves from this situation. First, the future of the wild population can be monitored, and actions taken for conservation. Coupled with this are all factors due to the variability of the natural environment. Second, the limitations in habitat provide limited access. With only a few access countries for the global LAL and TAL product, the political climate, geographical location, and regional regulations can limit or cut off supply of endotoxin reagent.
The second current challenge to endotoxin testing is the cost of instruments and reagents. Initially, endotoxin testing has a very low equipment cost. The original endotoxin test using the LAL reagent is the gelation method. The gelation method of endotoxin testing does not require any special equipment other than a suitable source of incubation. A large percentage of users throughout the world are using this method. However, a much greater wealth of information can be obtained from quantitative methods. Quantitative methods were developed to be used with absorbance readers to provide quantitative values to the amount

Journal of Applied Bioanalysis Expert Opinion Article

of endotoxin present. The transition requires instruments and installation services that often need to be sourced from the United States, Japan, or the European Union to the ROW (rest of world) countries [6].

The third current challenge to endotoxin testing is rigid guidelines in the face of evolving change. The three major pharmacopeial bodies, regulatory bodies such as the FDA and EMA, and ANSI/AAMI all publish guidelines, recommendations, and regulations on endotoxin testing. The regulations are essential to the uniform use and manufacture of the endotoxin testing reagents. However, the regulations do mean that innovations in testing methods often do not become a part of the guidance and regulations for years after the changes are proposed. One example of this is the recombinant Factor C (rFC) recombinant alternative to LAL. Although it was developed in the early 2000s and was accepted as compendial by the Ph.Eur., the USP and the JP have not followed in making recombinant LAL a compendial method of endotoxin detection [7]. Although this does lead to consistency in the testing methods, as the environment changes, needed changes in the endotoxin testing world often get delayed [8].

3.0 Opportunities

Challenges can do one of two things. They can expose inadequacies and raise a list of roadblocks, but they can also lead to the development of opportunities to grow and expand where previous methods had fallen short. Endotoxin testing, for the challenges that it currently faces, also has paths forward for innovation to overcome those challenges.

The first set of opportunities comes against the challenges of resource limitations. First, potential exists to farm horseshoe crabs in a controlled environment. The development of horseshoe crab aquaculture would provide two major benefits to the need for LAL/TAL. First, this would allow the populations of captive crabs to be controlled in ways that are impossible with wild populations. This would mitigate the effects of natural disasters and human fishing on the supply. Second, this would potentially allow populations of the crabs to be brought to locations that do not have access to naturally occurring crab populations [9].

Second, alternative endotoxin testing provides a completely controlled, synthetic reagent for endotoxin testing. One-factor synthetic alternatives utilize a synthetic version of the “Factor C” protein that binds to endotoxin. Three-factor synthetic alternatives utilize the complete cascade pathway found within natural LAL. The two major factors limiting the universal adoption of synthetic LAL are uncertainty in equivalency from the regulatory bodies, and the cost of the product compared to natural LAL. However, as confidence grows in the synthetic product, the regulatory bodies will continue to adopt the alternative method. The European Pharmacopoeia has already made one-factor alternatives compendial. As more products are manufactured, efficiency in manufacturing will increase, and prices will come down as well [10].

The second set of opportunities comes against the cost of reagents and instruments. Since the initial development of using absorbance readers to provide quantitative results, many iterations of readers have been made to optimize accuracy in measurements. These include traditional microplate readers, tube readers, and cartridge systems. New instruments have been developed with portability in mind. Using portable, rugged, instruments, quantitative testing can be provided to areas that only had access to qualitative testing [11]. Using the properties of polarized light, a newer generation of instruments can make absorbance readings with a reduced amount of reagent. This conservation leads to a greater usage efficiency of the volume of reagent [12].

Finally, the third set of opportunities comes as greater communication between the reagent manufacturers, users, and the regulatory bodies. Synchronization between all three parties is essential for the advancements in endotoxin testing. Recently, beneficial dialogue has occurred between the major pharmacopeia and the endotoxin manufacturers—especially in the advancements of synthetic reagents. Promising changes have been made, and the optimism is increasing to see a greater dialogue and cooperation between all parties involved [13].

4.0 Conclusions

Although LAL endotoxin testing is facing many challenges, it has opportunities to improve and overcome each of these challenges. From its origin as a qualitative gel clot method, to the current industrial standard for quantitative endotoxin testing, LAL testing has developed. These developments are not over, and future developments in LAL testing and recombinant LAL testing are on the horizon. These processes will allow endotoxin testing to grow in the future.

5.0 References

1. Sandle T. Historical Milestones and Industry Drivers in the Development of Recombinant Lysate for Bacterial Endotoxin Testing. Am Pharm Rev 23, 4-7 (2020).
2. Jacobson, Lawrence, Carmichael Ruth, Cieri Matthew. 2019 Horseshoe Crab Benchmark Stock Assessment and Peer Review Report. Atlantic States Marine Fisheries Commission (2019). http://www.asmfc.org/uploads/file/5cd5d6f1HSCAssessment_PeerReviewReport_May2019.pdf (Accessed March 30, 2023).
3. Akbar J, Shin P, Botton M, Gauvry G, Cheung SG, Laurie K. Conservation of Asian horseshoe crabs on spotlight. Biodivers Conserv 30, 253-256 (2021).
4. Schrading E. U.S. Fish and Wildlife Service Evaluation of the Atlantic States Marine Fisheries Commission Horseshoe Crab/Red Knot Adaptive Resource Management Revision (2022). https://www.fws.gov/media/us-fish-and-wildlife-service-evaluation-atlantic-states-marine-fisheries-commission-horseshoe (Accessed January 31, 2023).

Journal of Applied Bioanalysis Expert Opinion Article

5. Chen LK et al. The IUCN Red List of Threatened Species (2019). https://horseshoecrab.org/press/2019/07/IUCN-Red-List_Supplemental_Combined.pdf (Accessed January 31, 2023).
6. Wong D, Capper K, Guest M, Duncanson P. A strategic approach to optimizations of testing bacterial endotoxins. Eur Pharm Rev (2022). https://www.europeanpharmaceutical-review.com/article/173738/a-strategic-approach-to-optimisations-of-testing-bacterial-endotoxins/ (Accessed January 31, 2023).
7. Williams K. Examining Claims Accepted as Fact in LAL and rFC Comparison Studies. American Pharmaceutical Review (2020). https://www.americanpharmaceuticalreview.com/Featured-Articles/569468-Examining-Claims-Accepted-as-Fact-in-LAL-and-rFC-Comparison-Studies/ (Accessed March 30, 2023).
8. Akers J et al. Attributes for Alternative Tests. Am Pharm Rev Endotoxin Supplement (2020). https://www.americanpharmaceuticalreview.com/Featured-Articles/565951-Functional-Challenges-for-Alternative-Bacterial-Endotoxins-Tests-Part-1-Attributes-for-Alternative-Tests/ (Accessed March 30, 2023).
9. Tinker-Kulberg R et al. Horseshoe Crab Aquaculture as a Sustainable Endotoxin Testing Source. Front Mar Sci 7, 153 (2020).
10. FUJIFILM Wako Pure Chemical Corp. FUJIFILM Wako Pure Chemical Corporation Launches Recombinant Protein-Reagent "PYROSTAR Neo" for Endotoxin Detection. CMO CRO (2021). https://www.cmocro.com/news_detail/FUJIFILM_Wako_Pure_Chemical_Corporation_Launches_Recombinant/654184/index.html (Accessed January 31, 2023).
11. Tamura H, Reich J, Nagasoka I. Outstanding Contributions of LAL Technology to Pharmaceutical and Medical Science: Review of Methods, Progress, Challenges, and Future Perspectives in Early Detection and Management of Bacterial Infections and Invasive Fungal Diseases. Biomedicines 9(5) (2021).
12. FUJIFILM Wako Chemicals U.S.A. Corp. FUJIFILM Wako LAL Division Announces New Endotoxin Testing Services Partnership. FUJIFILM Wako Chemicals U.S.A. Corp, (2022). https://www.wakopyrostar.com/blog/kit-lal/post/new-endotoxin-testing-services-partnership/ (Accessed January 31, 2023).
13. Williams K. rFC offers many advantages for industry. Parenteral Drugs Association (2020). https://www.pda.org/pda-letter-portal/home/full-article/rfc-offers-many-advantages-for-industry (Accessed January 31, 2023).