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Response to the Guest Editorial "Scientific Studies and Interpretation"

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LETTER TO THE EDITOR-RESPONSE

Response to the Guest Editorial “Scientific Studies and Interpretation”

The article “Scientific Studies and Interpretation” was published as a *Guest Editorial* in Vol. 74, No. 5, September–October 2020 of the *PDA Journal of Pharmaceutical Science and Technology (PDA-JPST)*. This aforementioned article discusses the use of recombinant reagents for bacterial endotoxin testing and interpretation of scientific studies. The following addresses certain comments and statements that are inaccurate or could be considered misleading within the article by highlighting the currently available evidence for the comparability of recombinant reagents, *Limulus* amoebocyte lysate (LAL) and horseshoe crab (HSC) availability, and recent scientific evidence.

Evidence for the Comparability of Recombinant Reagents

“Scientific Studies and Interpretation” addressed the importance for evidence-based practice (EBP) and stated, “EBP necessarily relies upon the publication of peer-reviewed data, derived from robust experimental designs that incorporate the best scientific knowledge available, and appropriate interpretation of the results.” In support of this perspective, Table I presents a nonexhaustive list of peer-reviewed data published on the comparability between recombinant reagents and LAL for bacterial endotoxin testing.

These studies have all been published in reputable peer-reviewed journals (including *PDA-JPST*), and the samples tested include hundreds of different matrices. Additionally, a review article was published in *PDA-JPST* that summarizes the current state of the research as well as the regulatory and compendial status of the use of recombinant reagents for endotoxin testing (“Currently Available Recombinant Alternatives to Horseshoe Crab Blood Lysates: Are They Comparable for the Detection of Environmental Bacterial Endotoxins? A Review”, *PDA-JPST* – Bolden, et al., 2020). The authors of the articles listed in Table I include two different country government agencies, three of the top 20 pharmaceutical manufacturers, a consortium of 15

pharmaceutical companies, and only one of the articles was written or conducted by a manufacturer of bacterial endotoxin test assays (see Table II).

LAL Availability

Biomedical harvest in 2019 was almost equal to bait harvest for the first time ever. This harvest increased 25% over that in 2018. According to the 2019 Atlantic States Marine Fisheries Commission Review of the Interstate Fishery Management Plan (FMP) regarding HSC harvests: “Estimated mortality from biomedical use in 2019 represents the highest value in the time series both in numbers of crabs (a 30% increase from 2018) and as a percentage of total directed use mortality” (1). The two factors of increased harvest and increased mortality are worrying signs in that both occurred before the start of the SARS-CoV-2 epidemic. Further, the review notes that in 2019, the total biomedical mortality was almost double the FMP’s mortality threshold of 57,500 HSCs, which as stated in the report requires the board to consider management action. In fact, this threshold has been exceeded in 12 of the last 13 years, with >100,000 estimated HSC deaths in 2019 alone. Reinforcing this concern and highlighting the utility of recombinant factor C (rFC) as a viable and comparable replacement for traditional LAL is the 2018 review and perspective article by Maloney et al. published in *PLOS Biology*: “Saving the Horseshoe Crab: A Synthetic Alternative to Horseshoe Crab Blood for Endotoxin Detection.”

Surprisingly, the October guest editorial article suggests that the solution to the concerns surrounding the HSC population is the use of an HSC-derived proprietary product exclusively supplied by a single LAL reagent manufacturer. Different solutions other than a monopoly situation should be considered by members of a standard-setting body.

Recent Scientific Evidence and Testing Data

Mentioned in the October guest editorial is “Recent scientific evidence and testing data (Tsuchiya, 2020) have illustrated that there could be a significant problem with the recombinant reagents, specifically, failing to exhibit comparability to naturally sourced *Limulus* amoebocyte lysates.” It is

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TABLE I
Recent Peer-Reviewed Articles on the Use of Recombinant Reagents for Endotoxin Testing

1. Comparison of Limulus amebocyte lysate test methods for endotoxin measurement in protein solutions, *J. Pharm. Biomed. Analysis*—Chen, L. and Mozier, N. (2013)
2. Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides, *PMDA*—Kikuchi, Y., et al., (Part 1) (2017)
3. Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides, *PMDA*—Kikuchi, Y., et al., (Part 2) (2017)
4. Results of a harmonized endotoxin recovery study protocol evaluation by 14 BioPhorum Operations Group (BPOG) member companies, *Biologicals*—Bolden J., et al. (2017)
5. Application of recombinant Factor C reagent for the detection of bacterial endotoxins in pharmaceutical products, *PDA-JPST*—Bolden, J. and Smith, K. (2017)
6. Study on the Applicability of Recombinant Factor C Method for Detection of Bacterial Endotoxin, *China Pharmaceuticals*—Pei, Y., et al. (2019)
7. Application of a Recombinant Three-Factor Chromogenic Reagent, PyroSmart, for Bacterial Endotoxins Test Filed in the Pharmacopeias, *Biol. Pharm. Bull*—Muroi, M., et al. (2019)
8. Comparison of LAL and rFC Assays-Participation in a Proficiency Test Program between 2014 and 2019, *Microorganisms*—Piehler, M., et al. (2020)
9. Comparison of LAL and recombinant Factor C endotoxin testing assays in human vaccines with complex matrices, *PDA-JPST*—Marius, M., et al. (2020)
10. Comparison of bacterial endotoxin testing methods in purified pharmaceutical water matrices, *Biologicals*—Marius, M., et al. (2020)
11. Evaluation of recombinant factor C assay for the detection of divergent lipopolysaccharide structural species and comparison with Limulus amebocyte lysate-based assays and a human monocyte activity assay, *J. Med. Microbiol.*—Abate, W., et al. (2017)

important to note that both the evidence and data are from the same LAL manufacturer whose proprietary product is recommended as a solution to the HSC population/LAL supply concerns; however, the evidence and data provided are fundamentally flawed. The reference provided by the guest editorial is the 2020 Tsuchiya paper that only reviewed selected research articles from the field of endotoxin testing and did not include data from new or original experiments; yet it still suggested findings to discredit recombinant reagents. Further investigation into the article found that it was published in a “pay to publish” predatory journal (2) (Figure 1). Of note is that this article, of questionable quality and validity, was referenced in an editorial that purportedly supports, “an objective and balanced peer review process to ensure that (EBP) is accomplished without bias.”

Additionally, the testing data that the editorial refers to was again supplied by the same proprietary LAL reagent manufacturer mentioned previously, and at this time the data has **not** been peer-reviewed nor made publicly available. The testing data referenced focused on the evaluation of pretreatment water that has unknown microbiological, inorganic, and organic contents, such as the known LAL reactive materials

β -glucans and cellulosic residues. All of which could contribute to an unreliable determination of the endotoxin level in the samples (3). Further, pretreatment water is not relevant to pharmaceutical manufacturing and as such is not tested for endotoxin or total organic carbon in regular operations (4).

Overall, it is concerning that the editorial is promoting, “the publication of peer-reviewed data, derived from robust experimental designs that incorporate the best scientific knowledge available, and appropriate interpretation of the results,” while at the same time quoting to support their argument a paper published in a predatory journal and nonpublished pretreatment water test data from the same LAL manufacturer that is the sole supplier of the product the October editorial proposes as the solution to HSC population concerns.

Final Words

Whether the adoption of recombinant endotoxin testing will save the plight of the HSC is open for debate, but to neglect out of hand the body of data corroborating the comparability of recombinant reagents with LAL

TABLE II
Author Affiliations and Number of Scientific Publications Showing Comparability of Recombinant Reagent and *Limulus* Amebocyte Lysate-Based Endotoxin Testing in Pharmaceutical Manufacturing and the Associated Publisher

Author Affiliations	Number of Publications	Journal–Publisher
Government and University Organizations	4	<i>PMDRS</i> (2, 3)–Pharmaceutical and Medical Device Regulatory Science Society of Japan <i>China Pharmaceuticals</i> (6) <i>Journal of Medical Microbiology</i> (11)–Microbiology Society (Official journal of the Pathological Society of Great Britain and Ireland)
Pharmaceutical Manufacturers	5	<i>Journal of Pharmaceutical and Biomedical Analysis</i> (1) <i>PDA-Journal of Pharmaceutical Science and Technology</i> (5, 9)–Parenteral Drug Association (PDA) <i>Biologicals</i> (4, 10)–Journal of the International Alliance for Biological Standardization
Endotoxin Testing Laboratory	1	<i>Microorganisms</i> –MDPI (8)
Recombinant Assay Manufacturer	1	<i>Biological and Pharmaceutical Bulletin</i> (7)–Pharmaceutical Society of Japan

seems obstructionist and irresponsible. When Russell and Burch proposed the principles of replacement, reduction, and refinement (3Rs) in 1959, they called the eventual replacement of animal-based research, education, and testing the “ultimate goal” (5). The use of recombinant reagents for bacterial endotoxin testing further emphasizes, strengthens, and shows the continued importance of improving our global 3R practices for our future. It is important to note that despite all of the evidence, science, and research completed and continuously being conducted, there

is no movement for the outright replacement of the LAL assay reagents: LAL will always have its place in bacterial endotoxin testing. Proponents of furthering the use of recombinant reagents are only requesting equivalency based on the increasing volume of compelling data available for review. A prime example of this process of evaluation and consideration is the EDQM, who have taken the comprehensive data on rFC into consideration and, based on the demonstration of reliable endotoxin testing, introduced it into the European Pharmacopoeia as Chapter 2.6.32 (6).



Figure 1
Predatory journals.

To summarize, the reader should refer to the final quote of the article: “there are studies where data has been collected in an appropriate and scientific fashion and interpreted appropriately, and there are those that have not, there is no in between.” Indeed, there are numerous studies from non-reagent supplier sources using multiproduct, multicenter evaluations, hundreds of matrices, and multiple bacterial endotoxin species. These studies, which showed comparability of the recombinant reagents with LAL, were then published in peer-reviewed, reputable journals. This is in direct contrast to a proprietary LAL-reagent supplier providing an unpublished study using pretreatment water and publishing a single article in a predatory journal.

No one is arguing that the introduction of recombinant endotoxin testing reagents should not be supported by solid, scientific inquiry and rationale. However, the

article rejecting a new technology, regardless of the evidence presented, that can benefit our industry and the patients who rely on us just because it is new or contradicts the established practice benefits no one except those with a vested interest in keeping the status quo. The evidence is there, and it is now incumbent on all parties to act in the scientific and public interest.

Conflict of Interest Declaration

Brendan Tindall is an employee of bioMérieux.

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