This list is provided as a resource to find scientific data related to recombinant factor C (rFC) bacterial endotoxins tests. It is a bioMérieux-curated list of peer-reviewed articles related to factor C assays for the detection of bacterial endotoxins. Articles are not restricted to bioMérieux products and the list may not be exhaustive. It is intended for informational reference purposes.

<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>CITATION</th>
<th>AFFILIATION</th>
</tr>
</thead>
</table>

A metastudy demonstrating comparability of rFC to LAL. rFC is more sustainable for supply chains (not relying on animal source like LAL) and specificity (lack of Factor G pathway thus no false positives due to beta-glucans). The article includes a review of the current compendia and regulatory status of the recombinant technologies.


| **Eli Lilly and Company; Bristol Myers Squibb; University of California School of Medicine; European Directorate for the Quality of Medicines and HealthCare; American Association Of Pharmaceutical Scientists; Pfizer; Paul-Ehrlich-Institute; Roche-Genentech** |

This study compared an rFC assay with two LAL assays for environmental water testing to evaluate if the rFC assay could increase throughput while maintaining low rates of invalid results. The rFC assay was a good replacement for LAL as it performed similarly, improved batch-to-batch consistency and increased specificity and robustness.


| **Sanofi Pasteur** |

This study compared two rFC assays with two LAL assays for endotoxin detection in four vaccine samples. The rFC assays were suitable for detection of endotoxin and provided the advantage of higher specificity for endotoxin in samples containing glucans, making them suitable for the release of the tested products.


<p>| <strong>Sanofi Pasteur</strong> |</p>
<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>CITATION</th>
<th>AFFILIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>The reactivity of rFC to different LPS structures was evaluated and compared to LAL and MAT assays. rFC was determined to be a good replacement for the conventional LAL assay and correlated significantly with the IL-6 levels produced by a human monocyte cell line.</td>
<td>Abate, W. et al. 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. Journal of medical microbiology vol. 66,7 (2017): 888-897. doi:10.1099/jmm.0.005510</td>
<td>Centre for Biomedical Research, School of Biomedical and Healthcare Sciences, Peninsula Schools of Medicine and Dentistry, Plymouth University; Academic Unit of Ophthalmology, University of Bristol; Centre for Research in Biosciences, Faculty of Health and Life Sciences, University of the West of England</td>
</tr>
<tr>
<td>rFC was shown to be equivalent to LAL in quantifying endotoxins in different matrices and in range of detection for different endotoxins. The quantitation range of rFC was comparable to that of quantitative photometric LAL.</td>
<td>Loverock, B. et al. A recombinant factor C procedure for the detection of Gram-negative bacterial endotoxin. Pharmacopeial Forum 36 (2010): 321–329.</td>
<td>Lonza Walkersville, Inc.</td>
</tr>
<tr>
<td>Members of the BioPhorum Operations Group (BPOG) aimed to develop a harmonized protocol for endotoxin recovery. Consistent results were obtained between all methods (LAL and rFC) independent of product matrix, laboratory or endotoxin type.</td>
<td>Bolden, J. et al. Results of a harmonized endotoxin recovery study protocol evaluation by 14 BioPhorum Operations Group (BPOG) member companies. Biologica: journal of the International Association of Biological Standardization vol. 48 (2017): 74-81. doi:10.1016/j.biologica.2017.05.003</td>
<td>Eli Lilly and Company; Genentech; Bayer Healthcare Llc.</td>
</tr>
<tr>
<td>Endotoxin activity was evaluated using three LAL and three rFC assays. Comparable results were observed for rFC and LAL with purified LPSs and NOE, however, in the uncharacterized natural water samples reactivity was higher with LAL than rFC and was attributed to the presence of LAL-reactive materials.</td>
<td>Kikuchi, Y. et al. Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides. Pharmaceutical and Medical Device Regulatory Science 48,4 (2017): 252-260.</td>
<td>National Institute of Health Sciences; Pharmaceutical and Medical Device Regulatory Science Society of Japan; Japan Food Research Laboratories; M Labs Inc.; bioMerieux Japan Ltd.; Seikagaku Corporation; Lonza Japan Ltd.; FUJIFILM Wako Pure Chemical Industries</td>
</tr>
<tr>
<td>Endotoxin activity for three LAL and three rFC assays was further evaluated using purified LPS from additional strains. As with the outcome from the previous study, rFC was found to be comparable with LAL for BET.</td>
<td>Kikuchi, Y. et al. Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides, Part 2. Pharmaceutical and Medical Device Regulatory Science 49,10 (2018): 706-718.</td>
<td>National Institute of Health Sciences; Pharmaceutical and Medical Device Regulatory Science Society of Japan; Japan Food Research Laboratories; M Labs Inc.; bioMerieux Japan Ltd.; Seikagaku Corporation; Lonza Japan Ltd.; FUJIFILM Wako Pure Chemical Industries</td>
</tr>
<tr>
<td>The lot-to-lot reproducibility of the rFC assay was evaluated in dust samples with four commonly used extraction and assay media, and was demonstrated to be superior the one previously reported for LAL. The study also makes suggestions for developing a standardized methodology for the measurement of environmental samples with rFC.</td>
<td>McKenzie, J. H. et al. Evaluation of lot-to-lot repeatability and effect of assay media choice in the recombinant Factor C assay. Journal of environmental monitoring : JEM vol. 13,6 (2011): 1739-45. doi:10.1039/c1jem10035a</td>
<td>Biomedical Engineering and Biotechnology Program, University of Massachusetts; Department of Environmental Health, Harvard School of Public Health; Channing Laboratory, Harvard Medical School; Maryland Institute for Applied Environmental Health, School of Public Health, University of Maryland</td>
</tr>
<tr>
<td>A quick point-by-point commentary discussing the comparison studies of rFC and LAL: non-purified water is an inappropriate sample, beta-glucan blockers are insufficient, hypotheses on higher LAL reactivity are speculative, USP draft &lt;1085.I&gt; requirements for rFC are excessive and inappropriate. All points are explained in an easy to understand language.</td>
<td>Williams, K. Examining Claims Accepted as Fact in LAL and rFC Comparison Studies. American Pharmaceutical Review vol. 23,6 (2020).</td>
<td>bioMerieux</td>
</tr>
<tr>
<td>Paper discusses claims of endotoxin underestimation by rFC and that Factor C is the specific biosensor for endotoxin. Main points addressed are whether or not non-purified water is inappropriate sample for comparison and whether or not USP chapter &lt;1225&gt; should be limited to products tested for endotoxin. Claims are supported by comparison data from experimental studies.</td>
<td>Williams, K. LAL and rFC Comparison Study Caveats. American Pharmaceutical Review (2020).</td>
<td>bioMerieux</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>CITATION</td>
<td>AFFILIATION</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Shuanghuanglian Injection, a traditional chinese medicine. Several</td>
<td></td>
<td></td>
</tr>
<tr>
<td>batches were tested with two rFC reagents and results compared to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAL gel clot. rFC was found to be suitable for bacterial endotoxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>testing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>samples closer to 100% and with lower variations than LAL. Demonstrating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>that rFC is equivalent or superior to LAL.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interference and Low Endotoxin Recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rFC was found to specifically detect endotoxins in the drug,</td>
<td>Pei, Y. et al. Methodological study on the detection of bacterial endotoxin in fosaprepitant dimeglumine by recombinant factor C. Chin J Mod Appl Pharm, vol. 36,1 (2019): 1-4.</td>
<td>National Institutes for Food and Drug Control; Shandong Institutes for Food and Drug Control</td>
</tr>
<tr>
<td>fosaprepitant dimeglumine, and was unaffected by interference contrary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>to the LAL gel clot and chromogenic tests methods that provided</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-compliant results.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>detection, is shown to improve robustness in endotoxin testing of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>buffer components compared to LAL, enabling its use with complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>matrices.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>results. Six critical parameters of BET assays are discussed, amongst</td>
<td></td>
<td></td>
</tr>
<tr>
<td>other variation between LAL assays, the activity of LPS and variations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in standard curves.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Validation and Regulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presents a non-inferiority study design strategy to validate the use</td>
<td>Der, E. et al. Validation Strategy for New Recombinant Factor C Users. American Pharmaceutical Review (2022).</td>
<td>Roche Genentech; F. Hoffmann La Roche Ltd; Roche</td>
</tr>
<tr>
<td>of rFC for pharmaceutical water samples spiked with Reference Standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endotoxin.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChP; rFC was found to be equivalent to chromogenic LAL and, contrary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>to LAL, it was unaffected by beta-glucans, demonstrating that it is</td>
<td></td>
<td></td>
</tr>
<tr>
<td>suitable for bacterial endotoxin testing in biologics.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>to the Chinese Pharmacopoeia 2020 edition, and discusses the basic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>validation request for the application of rFC and its benefits.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>This article focuses on Case Study 7 and the ENDO-RS method for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>demasking endotoxin in pharmaceutical formulations exhibiting LER.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faderl, C. Expert view: Low Endotoxin Recovery (LER). European</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUMMARY</td>
<td>CITATION</td>
<td>AFFILIATION</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>Environmental and Supply Chain Sustainability</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This article discusses the efforts of the Ph. Eur. to end the use of rabbits in pyrogen testing and increase the use of synthetic alternatives such as rFC for the detection of bacterial endotoxins.</td>
<td>Charton, E. European Pharmacopoeia Approach to Testing for Pyrogenicity. American Pharmaceutical Review (2022).</td>
<td>European Pharmacopoeia Department, European Directorate for the Quality of Medicines &amp; HealthCare (EDQM), Council of Europe</td>
</tr>
<tr>
<td>rFC advocacy from an ecology perspective. This paper emphasizes the need to replace LAL by rFC to save an entire ecosystem based on the horseshoe crab (including shore birds) marking it clearly unsustainable.</td>
<td>Maloney, T. et al. Saving the horseshoe crab: A synthetic alternative to horseshoe crab blood for endotoxin detection. PLoS Biol 16,10 (2018): e2006607. <a href="https://doi.org/10.1371/journal.pbio.2006607">https://doi.org/10.1371/journal.pbio.2006607</a></td>
<td>Revive &amp; Restore; Wilson Sonsini Goodrich &amp; Rosati</td>
</tr>
<tr>
<td>Given industry’s recent focus on the sustainability of bacterial endotoxin testing (BET), here, AstraZeneca colleagues Miriam Guest, Karen Capper, Dennis Wong and Phil Duncanson share how they worked to establish a short-, mid- and long-term strategy to optimise BET across the global enterprise. They also explore some of the short-term benefits already realised through the company-wide rollout of their work.</td>
<td>Wong, D. et al. A strategic approach to optimisations of testing bacterial endotoxins. European Pharmaceutical Review (2022).</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td>This article addresses the question of LAL supply and the potential risks associated with reliance on a reagent derived from a single animal source. The use of rFC avoids potential supply shortages.</td>
<td>Williams, K and Tindall, B. The impact of supply chain risks and LAL reliance. European Pharmaceutical Review 3 (2020).</td>
<td>bioMérieux</td>
</tr>
<tr>
<td>Article identifies the main drivers of rFC adoption by pharmaceutical leaders and how the industry is actually creating changes in global regulatory acceptance.</td>
<td>Williams, K. Tipping point – what is driving the adoption of rFC for bacterial endotoxin testing? European Pharmaceutical Review</td>
<td>bioMérieux</td>
</tr>
<tr>
<td><strong>Automation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-automation of rFC reduced repetitive and time consuming sample dilution steps generating savings of more than 50% in operator working time.</td>
<td>Christler, A. et al. Semi-automation of process analytics reduces operator effect. Bioprocess and biosystems engineering vol. 43,5 (2020): 753-764. doi:10.1007/s00449-019-02254-y</td>
<td>Austrian Centre for Industrial Biotechnology; Institute of Bioprocess Science and Engineering, Department of Biotechnology, University of Natural Resources and Life Sciences Vienna</td>
</tr>
<tr>
<td><strong>General Discussion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Book chapter written by the inventors of rFC, amongst others Prof. Ding, describing the biotechnological efforts that led to the invention of rFC in a comprehensive way (from A to Z) listing the application areas for BET.</td>
<td>Li, P., Ho, and Ding, J. L. Biotechnology efforts to conserve horseshoe crabs through the development of recombinant factor C-based endotoxin test. Changing Global Perspectives On Horseshoe Crab Biology, Conservation and Management. (2015): 501-512. doi: 10.1007/978-3-319-19542-1_29.</td>
<td>Centre for Biomedical and Life Sciences, Singapore Polytechnic; Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore; Department of Biological Sciences, Faculty of Science, National University of Singapore</td>
</tr>
<tr>
<td>Mrs. Wimbish, Product Manager at Lonza summarizes the benefits of rFC for BET beginning with an emphasis on the need for an alternative method to detect endotoxins. Next, the regulatory hurdles to rFC adoption are discussed and closed with the conclusion that rFC will become “the go-to solution in the future”.</td>
<td>Wimbish, L. Advantages of recombinant Factor C based endotoxin testing. European Pharmaceutical Manufacturer (2015)</td>
<td>Lonza Walkersville, Inc.</td>
</tr>
<tr>
<td>Multicenter study to investigate the applicability of rFC to six representative varieties of pharmaceutical products. Results with rFC were within the acceptance range and met the interference test requirements, showing good applicability to the varieties of pharmaceutical products tested.</td>
<td>Pei, Y. et al. Study on the applicability of recombinant factor C method for detection of bacterial endotoxin. China Pharmaceuticals. 28,7 (2019): 1006–4931.</td>
<td>National Institutes for Food and Drug Control; Shandong Institutes for Food and Drug Control; Jiangsu Institutes for Food and Drug Control; Shanxi Institutes for Food and Drug Control</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>CITATION</td>
<td>AFFILIATION</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Discusses formulation differences between reagents (rFC, rLAL, LAL),</td>
<td>Sandle, T. Historical Milestones and Industry Drivers in the Development</td>
<td>Bio Products Laboratory Ltd.</td>
</tr>
<tr>
<td>divergence between pharmacopeias including development timeline, all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from the lab user perspective.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summary of the road to full rFC adoption, broken down into four</td>
<td>Ding, J. L. et al. Endotoxin Detection: The Four Pillars of rFC Adoption</td>
<td>Department of Biological Sciences, National University of Singapore; bioMérieux; Hyglos GmbH, Department of Food Science and Technology, National University of Singapore</td>
</tr>
<tr>
<td>pillars: 1. LAL comparability to RPT as first step towards the</td>
<td>in Lieu of LAL. American Pharmaceutical Review 23.6 (2020).</td>
<td></td>
</tr>
<tr>
<td>acceptance of alternative methods to detect endotoxins. 2. The</td>
<td></td>
<td></td>
</tr>
<tr>
<td>development of recombinant technologies ultimately leading to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recombinant Factor C. 3. rFC adoption roadblocks and 4. The future</td>
<td></td>
<td></td>
</tr>
<tr>
<td>full adoption of rFC based on increasing standardization.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study investigates the effects of low concentrations of</td>
<td>Schwarz, H. et al. Residual endotoxin contaminations in recombinant</td>
<td>Department of Molecular Biology, University of Salzburg</td>
</tr>
<tr>
<td>endotoxin contamination found in commercially available recombinant</td>
<td>proteins are sufficient to activate human CD1c+ dendritic cells. PloS one</td>
<td></td>
</tr>
<tr>
<td>proteins on human immune cells. The authors recommend screening</td>
<td>vol. 9,12 e113840 (2014) doi:10.1371/journal.pone.0113840</td>
<td></td>
</tr>
<tr>
<td>recombinant proteins for endotoxin impurities using LAL, rFC or a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>luciferase based NF-κB reporter cellular assay.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Information provided herein is for illustrative purposes only. This information cannot cover all situations or rules or policies, nor can use of the information guarantee compliance with every law or regulation. This communication makes no representations or warranties as to compliance with every law or regulation in all circumstances.