RECOMBINANT FACTOR C (rFC) ASSAY
CITATIONS LIST

LITERATURE RELATED TO RECOMBINANT FACTOR C (rFC) ASSAYS USED TO DETECT BACTERIAL ENDOTOXINS, 2010-2022.

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.

SUMMARY	CITATION	AFFILIATION
Comparability		
This study reports the validation of different pharmaceutical products with rFC. rFC was found to be equivalent or superior to LAL, in addition to improving assay specificity as it is unaffected by beta-glucans.	Bolden, J. and Smith, K. Application of Recombinant Factor C Reagent for the Detection of Bacterial Endotoxins in Pharmaceutical Products. PDA journal of pharmaceutical science and technology vol. 71,5 (2017): 405-412. doi:10.5731/pdajpst.2017.007849	Eli Lilly and Company
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.	Bolden, J. et al. Currently Available Recombinant Alternatives to Horseshoe Crab Blood Lysates: Are They Comparable for the Detection of Environmental Bacterial Endotoxins? A Review. PDA journal of pharmaceutical science and technology vol. 74,5 (2020): 602-611. doi:10.5731/pdajpst.2020.012187	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.	Marius, M. et al. Comparison of bacterial endotoxin testing methods in purified pharmaceutical water matrices. Biologicals : journal of the International Association of Biological Standardization vol. 67 (2020): 49-55. doi:10.1016/j.biologicals.2020.07.001	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.	Marius, M. et al. Comparison of Limulus Amoebocyte Lysate and Recombinant Factor C Assays for Endotoxin Detection in Four Human Vaccines with Complex Matrices. PDA journal of pharmaceutical science and technology vol. 74,4 (2020): 394-407. doi:10.5731/pdajpst.2019.010389	Sanofi Pasteur

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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.	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.0.000510	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
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.	Loverock, B. et al. A recombinant factor C procedure for the detection of Gram-negative bacterial endotoxin. Pharmacopeial Forum 36 (2010): 321–329.	Lonza Walkersville, Inc.
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.	Bolden, J. et al. Results of a harmonized endotoxin recovery study protocol evaluation by 14 BioPhorum Operations Group (BPOG) member companies. Biologicals : journal of the International Association of Biological Standardization vol. 48 (2017): 74-81. doi:10.1016/j.biologicals.2017.05.003	Eli Lilly and Company; Genentech; Bayer HealthCare Llc.
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.	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.	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
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.	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.	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
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.	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/c1em10035a	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
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 <1085.1> requirements for rFC are excessive and inappropriate. All points are explained in an easy to understand language.	Williams, K. Examining Claims Accepted as Fact in LAL and rFC Comparison Studies. American Pharmaceutical Review vol. 23,6 (2020).	bioMérieux
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 <1225> should be limited to products tested for endotoxin. Claims are supported by comparison data from experimental studies.	Williams, K. LAL and rFC Comparison Study Caveats. American Pharmaceutical Review (2020).	bioMérieux

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Comparison of rFC and LAL gel clot for bacterial endotoxin testing in Shuanghuanglian Injection, a traditional chinese medicine. Several batches were tested with two rFC reagents and results compared to LAL gel clot. rFC was found to be suitable for bacterial endotoxin testing.	Zhen, X. et al. Methodological study on the detection of bacterial endotoxin in Shuanghuanglian Injection by recombinant factor C. Lishizhen Medicine and Materia Medica Research, vol. 32, No.9 (2021): 2165-2167.	Hebei Institute for Drug and Medical Device Control
ENDOLISA and ENDOZYME II showed recovery rates of reference endotoxin samples closer to 100% and with lower variations than LAL. Demonstrating that rFC is equivalent or superior to LAL.	Piehler, M. et al. Comparison of LAL and rFC Assays-Participation in a Proficiency Test Program between 2014 and 2019. Microorganisms vol. 8,3 418. 16 Mar. 2020, doi:10.3390/microorganisms8030418	Microcoat Biotechnologie
Interference and Low Endotoxin Recovery		
rFC was found to specifically detect endotoxins in the drug, fosaprepitant dimeglumine, and was unaffected by intereference contrary to the LAL gel clot and chromogenic tests methods that provided non-compliant results.	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.	National Institutes for Food and Drug Control; Shandong Institutes for Food and Drug Control
ENDOLISA®, an assay based on high affinity LPS-binding and rFC for detection, is shown to improve robustness in endotoxin testing of buffer components compared to LAL, enabling its use with complex matrices.	Grallert, H. et al. EndoLISA®: a novel and reliable method for endotoxin detection. Nat Methods 8 (2011). https://doi.org/10.1038/nmeth.f.350	Hyglos GmbH.
Importance of data interpretation, especially for inconclusive results. Six critical parameters of BET assays are discussed, amongst other variation between LAL assays, the activitiy of LPS and variations in standard curves.	Delp, J. et al. The Truth of Endotoxin Values - Points for Consideration During Investigation of Aberrant BET Results. American Pharmaceutical Review 23,6 (2020).	Microcoat Biotechnologie
Validation and Regulation		
Presents a non-inferiority study design strategy to validate the use of rFC for pharmaceutical water samples spiked with Reference Standard Endotoxin.	Der, E. et al. Validation Strategy for New Recombinant Factor C Users. American Pharmaceutical Review (2022).	Roche Genentech; F. Hoffmann La Roche Ltd; Roche
Multicenter study to validate rFC based on Guideline <9101> of ChP. rFC was found to be equivalent to chromogenic LAL and, contrary to LAL, it was unaffected by beta-glucans, demonstrating that it is suitable for bacterial endotoxin testing in biologics.	Pei, Y. et al. Validation of a determination method for recombinant factor C of bacterial endotoxin. Chin J Biol, vol. 33,1 (2020): 76-79.	National Institutes for Food and Drug Control; Shandong Institutes for Food and Drug Control; Jiangsu Institutes for Food and Drug Control; Shanxi Drug Safety Risk Monitoring Center
Provides an introduction to the addition of rFC and micro-gel method to the Chinese Pharmacopoeia 2020 edition, and discusses the basic validation request for the application of rFC and its benefits.	Pei, Y. et al. Study on the Application of Supplementary Methods of Bacterial Endotoxin Test in Chinese Pharmacopoeia 2020 Edition. Chin J Mod Appl Pharm, vol. 39,6 (2022): 822–26. doi: 10.13748/j.cnki.issn1007-7693.2022.06.019	National Institutes for Food and Drug Control
Overview of the key points of the Technical Report No. 82 on LER. This article focuses on Case Study 7 and the ENDO-RS method for demasking endotoxin in pharmaceutical formulations exhibiting LER.	Faderl, C. Expert view: Low Endotoxin Recovery (LER). European Pharmaceutical Review 3 (2019).	bioMérieux

SUMMARY	CITATION	AFFILIATION
Environmental and Supply Chain Sustainability		
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.	Charton, E. European Pharmacopoeia Approach to Testing for Pyrogenicity. American Pharmaceutical Review (2022).	European Pharmacopoeia Department, European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe
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.	Maloney, T. et al. Saving the horseshoe crab: A synthetic alternative to horseshoe crab blood for endotoxin detection. PLoS Biol 16,10 (2018): e2006607. https://doi.org/10.1371/journal.pbio.2006607	Revive & Restore; Wilson Sonsini Goodrich & Rosati
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.	Wong, D. et al. A strategic approach to optimisations of testing bacterial endotoxins. European Pharmaceutical Review (2022).	AstraZeneca
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.	Williams, K and Tindall, B. The impact of supply chain risks and LAL reliance. European Pharmaceutical Review 3 (2020).	bioMérieux
Article identifies the main drivers of rFC adoption by pharmaceutical leaders and how the industry is actually creating changes in global regulatory acceptance.	Williams, K. Tipping point – what is driving the adoption of rFC for bacterial endotoxin testing? European Pharmaceutical Review | Volume 27, Issue 05	bioMérieux
Automation		
Semi-automation of rFC reduced repetitve and time consuming sample dilution steps generating savings of more than 50% in operator working time.	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	Austrian Centre for Industrial Biotechnology; Institute of Bioprocess Science and Engineering, Department of Biotechnology, University of Natural Resources and Life Sciences Vienna
General Discussion		
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.	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.	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
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".	Wimbish, L. Advantages of recombinant Factor C based endotoxin testing. European Pharmaceutical Manufacturer (2015)	Lonza Walkersville, Inc.
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.	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.	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

SUMMARY	CITATION	AFFILIATION
Discusses formulation differences between reagents (rFC, rLAL, LAL), mechanism of action, benefits of recombinant technologies, and divergence between pharmacopeias including development timeline, all from the lab user perspective.	Sandle, T. Historical Milestones and Industry Drivers in the Development of Recombinant Lysate for Bacterial Endotoxin Testing. 23 (2020): 4-7.	Bio Products Laboratory Ltd.
Summary of the road to full rFC adoption, broken down into four pillars: 1. LAL comparability to RPT as first step towards the acceptance of alternative methods to detect endotoxins. 2. The development of recombinant technologies ultimately leading to Recombinant Factor C. 3. rFC adoption roadblocks and 4. The future full adoption of rFC based on increasing standardization.	Ding, J. L. et al. Endotoxin Detection: The Four Pillars of rFC Adoption in Lieu of LAL. American Pharmaceutical Review 23,6 (2020).	Department of Biological Sciences, National University of Singapore; bioMérieux; Hyglos GmbH, Department of Food Science and Technology, National University of Singapore
This study investigates the effects of low concentrations of endotoxin contamination found in commercially available recombinant proteins on human immune cells. The authors recommend screening recombinant proteins for endotoxin impurities using LAL, rFC or a luciferase based NF-kB reporter cellular assay.	Schwarz, H. et al. Residual endotoxin contaminations in recombinant proteins are sufficient to activate human CD1c+ dendritic cells. PloS one vol. 9,12 e113840 (2014) doi:10.1371/journal.pone.0113840	Department of Molecular Biology, University of Salzburg

bioMérieux, Inc. • 100 Rodolphe Street • Durham, NC 27712 • U.S.A. • (800) 682-2666
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