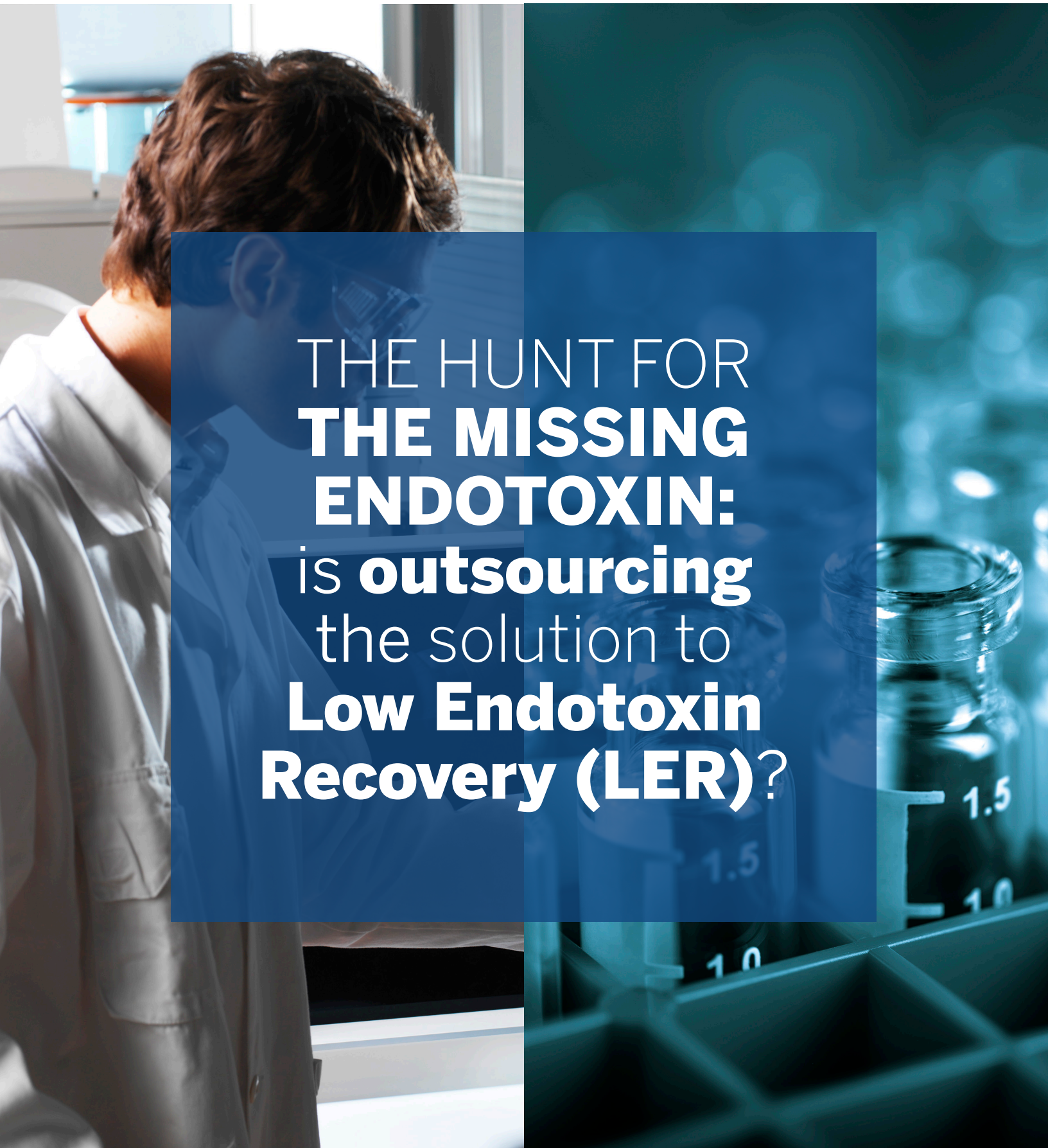
BIOMÉRIEUX



THE HUNT FOR **THE MISSING ENDOTOXIN:** is **outsourcing** the solution to **Low Endotoxin Recovery (LER)**?

This white paper reviews the causes contributing to low endotoxin recovery (LER), the impact it creates, as well as solutions which can rectify the issues that result. We also discuss the advantages and disadvantages of developing your de-masking protocols in-house, and how you can use the ENDO-RS® de-masking toolkit to make the process easier from beginning to end. Following this, we outline the pros and cons of outsourcing your de-masking project, what you should look for in a third-party company, and why you can and should trust bioMérieux with your de-masking protocol development.

INTRODUCTION

Without a shadow of a doubt, drugs are evolving. Consider, for example, how small molecules once dominated the pharmaceutical industry, yet today, there are increasing numbers of biologics such as antibodies and proteins on the market. To date, there are over 400 recombinant therapies approved by the FDA, demonstrating how biologics provide advantages including increased efficacy and specificity, while reducing toxicity and the risk of adverse side-effects.[1,2]

Unfortunately, the processes used to produce stable biologic formulations often present a specific problem, namely that of endotoxin detection. Endotoxins are lipopolysaccharide molecules (LPS) from gram-negative bacterial cell walls that can cause severe immune responses in patients, resulting in sepsis, and even death.[3]

Endotoxin detection is an integral part of the drug quality control process, and all sterile pharmaceutical and medical device products that come in contact with a patient's blood, or with cerebral or ocular fluids must be tested for endotoxins before their release. However, in some cases, the presence of masked endotoxins can render widely used endotoxin testing methods unreliable - a phenomenon known as 'low endotoxin recovery' (LER).[3]

We spoke to our expert, Dr. Christian Faderl, to find out more about masked endotoxins and how pharmaceutical manufacturers can effectively tackle the problem of LER.

MASKED ENDOTOXINS: UNDERSTANDING HIDDEN DANGERS

There are several methods currently available enabling testing for bacterial endotoxins: **limulus amebocyte lysate (LAL), tachypleus amebocyte lysate (TAL), and recombinant factor C (rFC).**

However, all of these methods rely on a protein known as Factor C, which binds with LPS molecules and sets off a reaction or cascade of reactions enabling endotoxins to be detected and measured. Significantly, Factor C is only activated by LPS in their natural, aggregated form.[3,4]

Manufacturing processes for biologics and final product formulations frequently require polysorbate surfactants to prevent protein aggregation, and chelating biological buffers to maintain an optimal pH.

These ingredients are essential to ensure that biologics are stable, and able to maintain their structure and function. However, they can also break down the aggregate structure of LPS, which can completely disperse into monomers within a solution. These dispersed monomers can no longer activate Factor C, and as such, remain undetected by endotoxin tests resulting in false negatives.[4-6]

Dr. Joseph Chen first reported LER at the PDA Annual Meeting in 2013. Dr. Chen showed that adding low levels of endotoxin to undiluted biologics could not be detected using the approved endotoxin testing methods. Since then, several further studies have demonstrated the effects of endotoxin masking and LER.[7-10]

"LER is a big safety risk for patients", says Dr. Faderl. **"If a product has a LER issue, the endotoxin is hidden, so you can't detect contamination during quality control. But masked endotoxins can still trigger an immune response."**

A recent study by scientists from the University of Salzburg has shown that masked endotoxins induced a response from human white blood cells, even when present at very low concentrations.[8]

ARE THERE MASKED ENDOTOXINS IN YOUR PRODUCT?

"It's vital to test your product for LER. If there are LER issues, they must be overcome by de-masking the endotoxin so you can conduct valid endotoxin tests during quality control and release testing," stresses Dr. Faderl.

Regulatory authorities are in concurrence with Dr. Faderl's sentiments, and companies that produce biologics are required to prove that they don't have issues with masked endotoxins or LER in the drugs they manufacture.[11]

"Hold time studies can show if you have an LER issue or not," explains Dr. Faderl, "To conduct the study, you spike your undiluted product with a reference standard endotoxin or control standard endotoxin, and then mimic your production process. Then, you let it stand at room temperature for a certain time, usually seven days. During this time, you test for endotoxins at certain time points, for example, directly after spiking, after four hours, six hours, one day, three days, and then seven days. If you can detect all your endotoxin at

every time point within the 50-200% endotoxin recovery range, you don't have an LER issue. But if your endotoxin recovery is less than 50% for two time points in a row, you have a problem."

Manufacturers who have issues with LER need to find out what is causing the problem, and develop a solution for de-masking the endotoxins in order that they can be detected by bacterial endotoxin tests, ensuring reliable quality control. A short-term solution could be to inject the drug product into rabbits and see if they experience an immune response (known as the Rabbit Pyrogen Test).

However, this is not a suitable long-term solution; the industry is trying to move away from animal testing, and instead use chemical tests or biological assays for analytical measurements.[3]

DEVELOPING DE-MASKING PROCESSES IN-HOUSE

Endotoxin masking is the result of chemical interactions that are specific to both your drug formulation and manufacturing processes.

Depending on the drug, there may well be a simple solution; for example, changing the sample container or adding magnesium chloride before bacterial endotoxin testing. However, this is only the case in around 5% of projects.

For the vast majority of projects, a process of understanding the molecular interactions causing LER, and then figuring out how de-masking the endotoxins can be achieved is required. The recently published PDA Technical Report No. 82 on LER outlines recommended procedures for the analysis and mitigation of endotoxin masking, commonly leading to LER in biologics.[5,12]

"You need a lot of knowledge, experience, and resources to overcome LER," says Dr. Faderl, **"It is not easy, because what you need to do depends on your drug product, and it also depends on what is triggering the LER. You need to know how to set up experiments and what to add to create a de-masking method that is effective, robust, and can be used in your daily quality control processes. It takes at least two technicians working full time to do all the experiments, and this can cost a lot of money."**

The advantages of developing endotoxin recovery processes in-house are the convenience and independence that come from in-house operations, and the peace-of-mind that the method you develop will work in your quality control processes and your laboratories.

However, the de-masking procedure development process requires a specialized skill set and particular resources, not the least of which is a team capable of performing the work. It is a relatively new area, and as a result, most people that are specialized in endotoxin testing are not necessarily familiar with endotoxin recovery.

Of course, once you have invested in these resources, you will have them in-house and available to you. Nonetheless, many manufacturers underestimate the time and investment required to develop the necessary in-house capabilities.

ESTABLISHING IN-HOUSE PROTOCOLS WITH ENDOTOXIN RECOVERY KITS

If you have access to the resources needed to develop your de-masking protocols in-house, you can use an endotoxin recovery kit such as the ENDO-RS® from bioMérieux to make the process easier and even more reliable.

Developing de-masking protocols using the ENDO-RS® was included in PDA technical report no. 82, as Case Study 7: Evaluation of an Endotoxin Demasking Protocol, the application of the ENDO-RS methodology developed by Hyglos (now bioMérieux).[12-14]

ENDO-RS® is a unique tool kit for sample preparation for bacterial endotoxin tests that addresses the issue of masked endotoxins. It uses a biochemical method that is both innovative and efficient for complete endotoxin recovery in biopharmaceutical formulations, and contains surfactants and chelating agents, while being independent of storage time and endotoxin concentration.

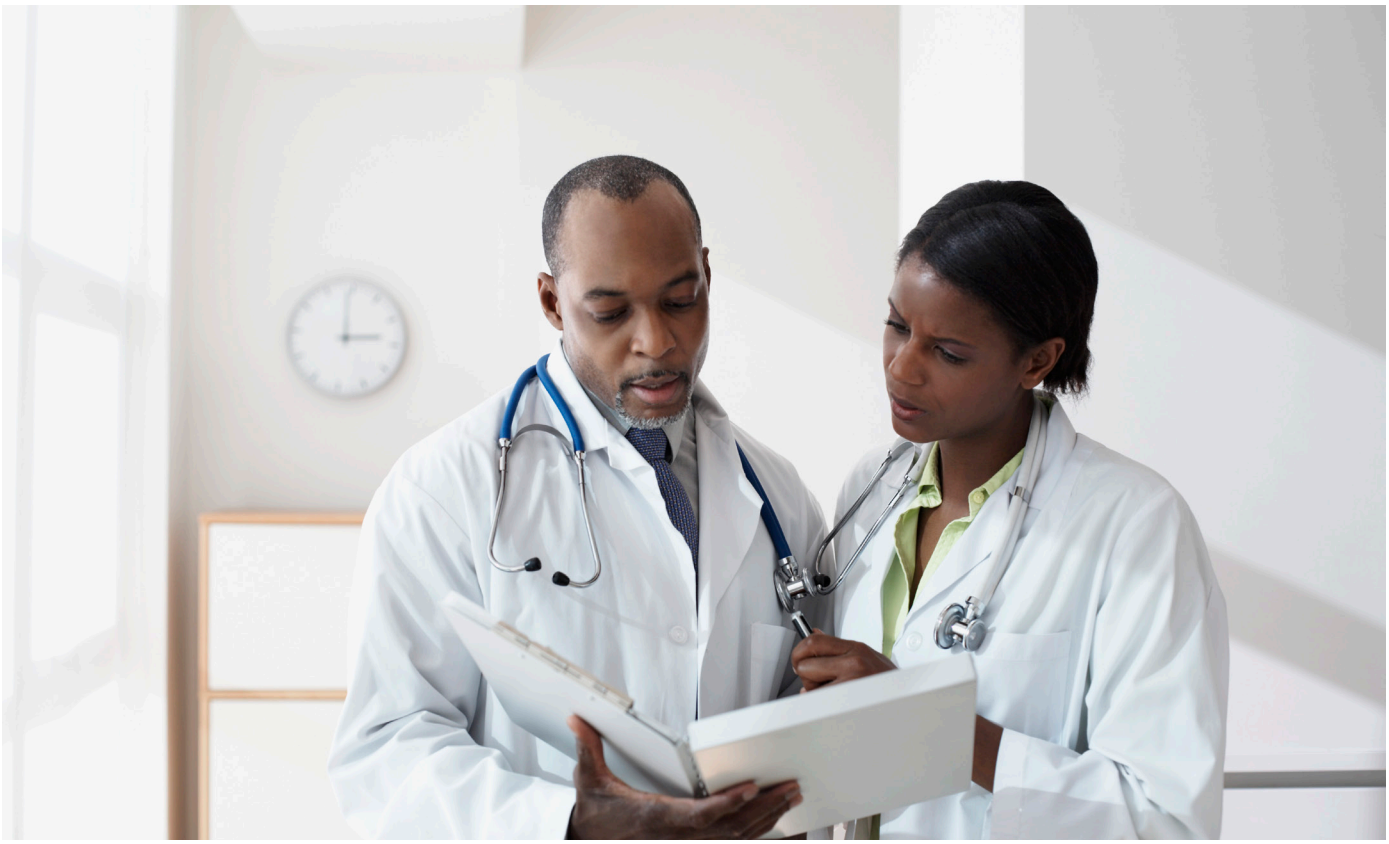
Combining ENDO-RS® and the ENDOLISA® bacterial endotoxin test provides accurate determination of endotoxins in biopharmaceuticals containing surfactants (e.g., polysorbate). It is also worth mentioning that the ENDO-RS® can also be combined with other bacterial endotoxin tests for complete flexibility.[12,15]

Case Study 7 used ENDO-RS® reagents in a sample affected by LER to de-mask endotoxins before endotoxin testing using LAL. The results showed that the ENDO-RS® was able to reliably de-mask the endotoxins in the sample, allowing them to be detected using LAL.

The de-masking procedures were repeated on different days by different operators, and across 21 runs, the variance in endotoxin recovery was 20%. The case study experiments explored the robustness of the kit by varying a range of conditions, including incubation time, vortex time, temperature, drug products, bacterial endotoxin testing protocols, and sources of LPS.

They found that after applying the ENDO-RS® demasking protocol, endotoxin recoveries were within the valid range for all the conditions tested, and were able to conclude that using the ENDO-RS® kit ensures functional endotoxin recovery, and it furthermore removes the risk of false-negatives by bacterial endotoxin tests.[12]

SOLVING LER ISSUES BY OUTSOURCING DE-MASKING PROCESS DEVELOPMENT



If you do not have the resources to develop de-masking protocols, outsourcing your process development can be a time and cost-effective way to overcome your LER problems. What's more, it allows you to bring a knowledgeable team to the project, without all the costs of developing such skills in-house.

As biopharmaceutical formulations differ in concentrations and compositions, an optimized de-masking protocol is needed for each drug formulation. "De-masking is always a research project," says Dr. Faderl.

“If you have no experience, you won’t know where the project is going, or how long it will take. An experienced company, on the other hand, will foresee problems and important factors that you may not have thought of.”[5]

bioMérieux’s ENDOXPERTS™ Endotoxin Services unit offers specialized method development and optimization services for LER, including the application of their specialized ENDO-RS technology. bioMérieux’s ENDOXPERTS have been running de-masking projects since 2015, completing 5-10 projects per year.

“We began working on de-masking projects shortly after the problem of LER was discovered, so we have the most experience in the market so far, and we have worked with leading pharmaceutical companies developing validated methods for LER that meet regulatory requirements,” asserts Dr. Faderl.[16]

“When you work with us, we discuss the product, the formulation you are using, and the hold-time studies you have done. Then we analyze the product formulation in our laboratories at bioMérieux,” says Dr. Faderl, who estimates that this root cause study takes around 1-2 months. “Once we fully understand your product and what is causing your LER issues, we agree on a plan for the de-masking project.”

bioMérieux’s processes are based upon a deep understanding of endotoxins and LER, and the experiments needed to develop the de-masking process are conducted by bioMérieux, a process that takes 6-9 months.

At the end of the development stage of the project, bioMérieux delivers a stable de-masking protocol that is optimized for your drug product. Their portfolio of LER solutions includes modifications such as changing containers, pH, or mixing procedures; adding cations such as Mg2+; adding dispersants such as pyrosperse; and other treatments, including the addition of enzymes or organic solvents.

BIOMERIEUX: COMPREHENSIVE DE-MASKING SERVICES THAT SUIT YOUR NEEDS

Once your third-party company has developed a de-masking protocol for your drug product, it is vital to ensure that the procedure works for the quality control processes of your in-house laboratories. While some third-party laboratories merely conduct the relevant experiments, before handing over the results for you to analyze and apply in your operations, bioMérieux provides a complete and comprehensive method transfer.

Once the de-masking process is validated, bioMérieux method transfer and training takes 1-2 months. Following this, full support from bioMérieux is on-hand to ensure the de-masking protocols work well in-house, and slot seamlessly into your existing quality control procedures.

bioMérieux's ENDOXPERTS™ bring the skills and knowledge of their dedicated team and the assurance that your de-masking protocols will work well in-house, without the costs associated with developing your personnel. What's more, bioMérieux's experience in LER and de-masking solutions ensures that the project will be completed as quickly, concisely, and cost-efficiently as possible.

The chemicals used in the manufacture of biologics can mask endotoxins, resulting in LER by endotoxin detection assays. Masked endotoxins still elicit immune responses which can be dangerous for patients. Companies that manufacture biologics must, therefore, prove that their bacterial endotoxin testing processes are not only reliable, but that they do not have a problem with masked endotoxins and LER.

De-masking endotoxins can be a complicated procedure, and developing processes to reliably de-mask endotoxins requires a highly specialized skill set. De-masking processes can be developed and validated in-house, but outsourcing endotoxin unmasking process development can save time and money, while also resulting in a safer and more reliable method.

However, it's essential to only outsource to companies with experienced LER laboratories offering proven results. bioMérieux has more experience with LER than anyone else in their field, and they have developed the only endotoxin recovery kit on the market. Furthermore, they provide a comprehensive service for LER, including routine testing and de-masking process development for transfer in-house.

"Anyone who needs assistance with an LER issue should contact us at bioMérieux, and we will work together with them to overcome their problem and ensure they have reliable bacterial endotoxin testing protocols," says Dr. Faderl.

REFERENCES AND FURTHER READING

1. Sanchez-Garcia L, Martín L, Mangues R, Ferrer-Miralles N, Vázquez E, Villaverde A. 'Recombinant pharmaceuticals from microbial cells: a 2015 update' Microb Cell Fact. 2016; 15: 33.

2. Morrow T, Felcone LH. 'Defining the difference: What Makes Biologics Unique' Biotechnol Healthc. 2004 Sep; 1(4): 24-26,28-29.

3. Williams K. 'Endotoxin Detection and Control in Pharma, Limulus, and Mammalian Systems' Springer, 2019.

4. Wang C, Nelson T, Chen D, Ellis JC, Abbott NL. 'Understanding Lipopolysaccharide Aggregation and its Influence on Activation of Factor C' J. Colloid Interface Sci. 2019; 552: 540-553.

5. http://www.biopharminternational.com/overcoming-low-endotoxin-recovery

6. Reich J, Tamura H, Nagaoka I, Motschmann H. 'Investigation of the kinetics and mechanism of low endotoxin recovery in a matrix for biopharmaceutical drug products' Biologicals. 2018; 53: 1-9.

7. Chen, J. "Low Endotoxin Recovery in Common Biologics Products." Presented at the 2013 PDA Annual Meeting, Orlando, Fla., April 2013.

8. Reich J, Lang P, Grallert H, Motschmann H. 'Masking of endotoxin in surfactant samples: Effects on Limulus-based detection systems' Biologicals. 2016; 44(5): :417-422.

9. Schwarz H, Gornicec J, Neuper T, Parigiani MA, Wallner M, Duschl A, Horejs-Hoeck J. 'Biological Activity of Masked Endotoxin' Scientific Reports. 2017; 7: 44750.

10. https://www.americanpharmaceuticalreview.com/Featured-Articles/239898-Low-Endotoxin-Recovery-A-Brief-Overview/

11. Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers, U.S. Food and Drug Administration, June 2012 https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-pyrogen-and-endotoxins-testing-questions-and-answers

12. "Low Endotoxin Recovery" PDA Technical Report No. 82 2019.

13. https://biomerieuxdirect.com/industry/c/ENDO-RS®/p/609065

14. https://en.a3p.org/endotoxin-masking/

15. https://biomerieuxdirect.com/industry/c/ENDOLISA®/p/609033

16. https://www.biomerieux-industry.com/products/endoxperts-endotoxin-services

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