# The Expanding Need for Endotoxin Testing

**Pyrogen testing was borne out of the need to screen medical devices and pharmaceuticals for molecules (pyrogens) that could initiate an intense reaction mimicking septic shock. These pyrogens can survive steam sterilisation. They are endotoxins from bacteria that survive beyond the sterilisation from the microorganisms themselves. The most potent of these pyrogens is the lipopolysacaride (LPS) molecules of gram negative bacterial. In vivio pyrogen testing was replaced by an endotoxin-specific test using a protein cascade found in the hemolymph of the horseshoe crab Limulus polyphemus. The Bacterial Endotoxin Test has become synonymous with QC testing for pharmaceutical products. Since the acceptance of the gel clot technique as a compendial test in 1980, endotoxin testing has become a crucial anchor for the expansion and development of safe pharmaceuticals and medical devices. Although much has changed in the 40+ years, the invaluable benefit of endotoxin testing has only grown. With the recent development, and compendial acceptance anticipated?, sustainable testing reagents have only ensured the permanency of the Bacterial Endotoxin Test for the next generation of pharmaceutical and medical advancements. In this editorial, I will provide an overview of the areas that are experiencing growth in their need of endotoxin testing.**

**Pharmaceutical Injectables**

The Bacterial Endotoxin Test is primarily focused on testing pharmaceutical injectables for product release. The guidelines provide guidance on calculating endotoxin limits allowing for concrete endotoxin limits to be determined. Since the 1980s, the BET has been well established as a requirement for injectables (USP <85>), and so most manufacturers of pharmaceuticals will be well established in endotoxin testing. The primary focus for these clients will be to transition to improved reagents due to its sensitivity or endotoxin specificity. FUJIFILM Wako provides reagents that are formulated to be endotoxin-free using large amounts of b-1, 3-glucan to saturate the protein (Factor G) found in the reagent that reacts to these molecules. This renders the LAL reagent to be endotoxin specific.

However, in recent years, with the withdrawal of the FDA's definite guidelines on sampling requirements, as well as the increase in customised drug products that are manufactured in small batches, new and expanded requirements have been created for the monitoring and control of the endotoxin manufacturing process and raw materials. Emphasis is placed on more continuous monitoring; allowing for sampling numbers to be lowered for end-product testing. Among contract drug manufacturers, often one form of endotoxin testing will be the standard practice, but they may be looking to expand their testing to different methods to better suit the needs of their customers.

As a result, rapid, affordable, quantitative data at the point of test has gained in popularity among pharmaceutical manufacturers. The primary focus is on the compliant, end-process testing that the reagents allow. However, a new market opening will be for the monitoring that rapid, economical testing provides.

For raw materials, excipient manufacturers generally have set endotoxin limits set by pharmacopeial monographs. However, API manufacturers, especially if they are new technologies such as cell and gene therapies, will often not have endotoxin limits. These areas are becoming more familiar with the endotoxin level needs of their clients and are a good market for adopting endotoxin testing.

The products in the pharmaceutical industry that are well established or expanding in their need of endotoxin testing are active pharmaceutical ingredients (API) manufacturing, excipient production, production and monitoring of sterile water for injection, expanded need for in-process product monitoring, contracted drug manufacturing, raw materials, injectable veterinary pharmaceuticals, PET tracers, and custom medical devices.

**Dialysis Devices and Solutions**

For Dialysis products, the dialysis equipment itself being endotoxin from the manufacturer's site is imperative. For manufacturers of dialysis equipment, tubing, grafts, catheters, membranes, and replacement fluids, as medical devices, it is well known and accepted that these will comply with endotoxin testing. Any manufacturer of these devices will be performing endotoxin testing.

A special need for developers of dialysis membrane tubing, whether cellulose or synthetic, will be for endotoxin testing of the membrane permeability to endotoxin as this is a critical factor in the amount of endotoxin reaching the bloodstream.

However, the primary focus at the forefront is endotoxin levels of dialysis water. Traditionally, dialysis water was held to a more lenient standard than WFI and is typically produced onsite. However, increased emphasis on the purity of the dialysate in addition to the permeability of the dialysis membrane has led to the definition of ultrapure dialysate with more stringent endotoxin limits. Practically, this has led to the implementation of WFI-quality filtration systems onsite. However, this has led to the need for an ability to effectively test microbial and endotoxin levels in the filtration systems to comply with the endotoxin levels as well as mitigate the growth of biofilms in the water production.

The release of ANSI/AAMI/ISO 23500-3:2019 was a regulatory push to bring all dialysis water to a uniform standard rather than being an advisory recommendation.

These guidelines push for monitoring of the water produced for dialysis for endotoxin testing, using a product such as the

aBET system or the gel clot single test for low volume, low-cost testing.

Manufacturers of dialysis equipment are targets for BET adaptation as well as any dialysis center that filters their water onsite.

The dialysis devices and solutions well established or expanding in their need of endotoxin testing include dialysis water/ultrapure dialysis water/buffers and solutions, cellulose acetate membranes/tubing/synthetic tubing, heparin and enoxaparin solutions/blood thinners, drain bags and lines, catheters and grafts, and water treatment systems.

For a client performing testing for environmental samples, the AAMI guidelines put an emphasis on the need for a rapid test method. Traditionally, the LAL test takes 60 minutes for incubation to occur. However, a reader such as the αBET endotoxin detection system can provide results in approximately 15 minutes for monitoring purposes.

**Testing Synopsis: Cell and Gene Therapeutics**

Currently, there are no binding endotoxin limits for cell and gene therapy. However, the FDA has released a 2019 draft guidance on "Setting Endotoxin Limits during Development of Investigational Oncology Drugs and Biological Products." Although not binding, the advice has the potential to become binding in the future. Specifically for Cell and Gene Therapy, it gives the following recommendations.

For early clinical products, all agents that are going to become a part of an investigational drug, including cell and gene therapy products, should have endotoxin levels within accepted BET requirements. Although not required, screening of these ingredients will ensure the safety and and purity of the final product.

For late-stage clinical development, endotoxin limits should be systematically validated and implemented to ensure that by the time of the marketing application, the entire validated procedure is in place. This limit should reference USP General Chapter <85>'s requirements for the risk and exposure that this investigational drug and concurrent additional administrations of ancillary drugs., to ensure that the patient dose remains in compliance of the pharmaceutical threshold pyrogenic dose.

Initially, performing a risk assessment and assigning a preliminary endotoxin will allow for the adoption of monitoring to take place during the early clinical development stage to fulfil these FDA recommendations. This should lead to the full validation of the endotoxin testing procedure being completed during the late-stage clinical development. As the reagent manufacturer, FUJIFILM Wako provides our clients with validation guides and technical guidance through this process.

Since monitoring is all that is recommended during this procedure, clients may find the aBET system appealing. The client could then continue to use the aBET system for their compliant testing or use the same reagent in transitioning over to more large-volume testing.

Some of the major products in the growing development of cellular therapeutics that are well established or expanding in their need of endotoxin testing are cell products, excipients, viral particles, DNA plasmid material, and cell culture media.

The main concern for these products is the complex molecules found in the API's and excipients of these products. The guidance does allow risk assessments to provide alternatives to traditional sampling and testing when it is shown that all the ingredients coming together are endotoxin free and the process is under control. Apart from that, various solutions such as heat treatment, dilution, and a treatment such as Predictive Oncology's EndoPrep can mitigate interferences from proteins and potential endotoxin masking in these complex products.

**Conclusion**

As the need for the safety screening of new pharmaceutical products and medical solutions becomes apparent as the clinical trials approach, the need for endotoxin testing of the products becomes imperative?. A revolutionarily beneficial product is not beneficial if it is not also safe from environmental contamination. Thankfully, the Bacterial Endotoxin Test is readily implemented in nearly any laboratory situation. The availability of traditional and simple qualitative testing, effective and resilient quantitative test reagents, as well as sustainable recombinant reagents provide an endotoxin solution for every need. Please reach out to FUJIFILM Wako's LAL division for support and guidance through every step of the way of your testing needs.

**REFERENCES**


1. USP. Bacterial Endotoxin Test, Chapter <85>
2. ANSI/AAMI/ISO. Preparation and Quality Management of Fluids for Haemodialysis and Related Therapies, 23500-3:2019.
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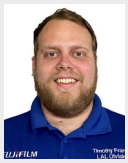


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