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Endotoxins Analysis - Medistri

🚀 Endotoxins Analysis

Bacterial endotoxins can trigger severe inflammation and death if they enter the bloodstream, cerebrospinal fluid, or intraocular fluid. Accurate detection of endotoxin levels in medical products is therefore an important part of the sterility testing process. Evaluation of bacterial endotoxin pyrogens is included in the International Organization for Standardization (ISO)'s new standard for microbiological methods.

Endotoxins are molecules produced by gram-negative bacteria that can contaminate medical products and have lethal consequences. They are shed by bacteria such as Escherichia coli (E. coli) and Salmonella during cell growth, cell division, and particularly in cell death, and are a type of pyrogen – a fever-causing substance. These molecules can have severe consequences if they enter the bloodstream; overexposure to endotoxins has been associated with severe inflammatory responses, septic shock, and death. It is vital that injectable medicines and implantable medical devices undergo endotoxin testing before use to ensure patient safety.

Medical devices are essential tools for diagnosing, monitoring and treating a variety of conditions. Importantly, they identify potential sources of microbial contamination and assess their risk to patients. However, the safety and efficacy of these devices can be compromised if they are not appropriately designed, manufactured, maintained and validated.

Endotoxin validation testing provides a more robust assessment of the test method. Briefly, inhibition and enhancement testing are performed using multiple dilutions/ diluents simultaneously. The results are then assessed to see which method generates the results closest to the ideal spike recovery percentage and sample pH.

Validation is important for several reasons:

1. **Accuracy:** Validation ensures that the test method is accurate and can provide reliable results. This is crucial in endotoxin testing as inaccurate results can lead to incorrect conclusions about the presence or absence of endotoxins.
2. **Reliability:** A validated method is reliable and can consistently produce the same results under the same conditions. This is important in ensuring the consistency of test results over time.
3. **Robustness:** Validation assesses the robustness of the test method, i.e., its ability to remain unaffected by small variations in method parameters. This ensures that the test method is stable and can withstand minor changes in the testing environment or procedure.
4. **Compliance with Regulatory Requirements:** Validation is often a regulatory requirement. Regulatory authorities require validation to ensure that the test methods used are suitable for their intended purpose and can provide accurate and reliable results.
5. **Risk Mitigation:** Validation helps to identify and mitigate risks associated with the test method. It allows for the identification of potential issues that could affect the accuracy or reliability of test results, enabling proactive measures to prevent such issues.

All types of samples can be tested: raw materials, finished products, sample dialysis, medical and surgical equipment. The method used at Medistri is kinetic colorimetry.

The kinetic colorimetric method is a type of endotoxin testing that is based on measuring the color developed by the chromophore released from a chromogenic substrate by the reaction of the endotoxin with the lysate. This method is a photometric assay.

In this method, a sample is mixed with lysate. In the presence of endotoxins, the lysate reacts with the endotoxin, resulting in a chromophore release from a chromogenic peptide2. The onset time needed to reach a predetermined absorbance of the reaction mixture is recorded by a microplate reader. Also, this method can be carried out by the chromogenic endpoint method or by the kinetic chromogenic method. The kinetic chromogenic method is based on measuring the color of the test sample at different time intervals after adding LAL reagent containing the colored substance.

The European Law for Medical Devices follows the MDR (Medical Device Regulation) and it replaced the MDD (Medical Devices Directive) in 2021. This change has been put in place to protect the health and safety of European Union citizens - ensuring that manufacturers produce safe products for Europeans. This includes the devices themselves as well as any medical device software (MDS).

The requirement for validation in endotoxins analysis has evolved over time. Initially, the Limulus Amebocyte Lysate Test (LAL) was proposed for determining endotoxins, and guidelines were issued in the Federal Register in January 1980. These guidelines were revised and reissued in 1983.

However, with the advent of the Medical Device Regulation (MDR), more stringent requirements have been put in place. It is now required to demonstrate that the test sample does not interfere with the ability to detect endotoxins. This is accomplished with the positive product control (also called the spike recovery) for the kinetic test methods, and with a separate inhibition and enhancement assay for the gel-clot method.

👉 This shift towards requiring validation for endotoxins analysis is part of a broader trend towards ensuring safety and efficacy in medical devices and pharmaceuticals. The goal is to ensure that endotoxin levels are accurately measured, which is crucial for patient safety.

The United States Pharmacopeia proposed a new Chapter < 86 >, which offers additional information on Bacterial Endotoxins Tests (BETs) using non-animal derived reagents. It has been published as a General Announcement in advance of public consultation.

This new standard was proposed by the committee earlier this year. This was based on the its evaluation of whether animal-free reagents could be used in addition to current methods for endotoxin testing utilising animal-derived reagents.

This new chapter also includes methods for using several reagents, including recombinant Factor C (rFC) and recombinant cascade reagents (rCR). It offers information for manufacturers of new and established biopharmaceuticals on ways to incorporate them into their quality testing.

Here's the included information in the proposed Chapter <86> Bacterial Endotoxins Test Using Recombinant Reagents:

- It's the user's responsibility for reviewing a supplier's primary validation package.
- Product suitability verification for use in testing products or materials (referencing <1226> Verification of Compendial Procedures)
- There's a possibility that regulatory authorities may require supplemental data prior to acceptance.

The International Organization for Standardization (ISO) has published its new standard Sterilization of health care products — Microbiological methods — Part 3 Bacterial endotoxin testing (ISO 11737-3:2023). The document contains requirements and guidance for testing for bacterial endotoxins.

👉 This includes products that must be non-pyrogenic based on either intended use or non-pyrogenic label claim, or both.

ISO 11737-3:2023 includes general criteria for determining bacterial endotoxins on or in health care products, components or raw materials using bacterial endotoxins test (BET) methods, using amebocyte lysate reagents. Only Gram-negative BET using amebocyte lysate reagents from Limulus polyphemus or Tachypleus tridentatus are covered.

By following these standards, manufacturers can demonstrate that their medical devices meet the safety and performance requirements of different markets and regulators.

🎯 To learn more about Medistri's Endotoxins Analysis, visit on our website here or directly contact our team at contact@medistri.swiss.

- The Medistri Team

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