Overlap and Differences Between Bacterial Endotoxin Testing and Extractables and Leachables Testing

In the world of pharmaceutical and medical device manufacturing, there are various quality control tests that may be required for distinct product types from separate regulatory agencies. With next generation medical device manufacturing, such as combination drug devices, the line between Bacterial Endotoxin Testing (BET) and Extractables and Leachables (E&L) Testing may become blurred. In fact, some may find considerable overlap between the goals of these methods with regards to product and patient safety.

Bacterial Endotoxin Testing: Background, Purpose and Testing Methodology

Bacterial Endotoxin Testing (BET) is performed to detect the presence of bacterial endotoxin in a drug product or medical device. Endotoxins, or lipopolysaccharides (LPS), are a type of pyrogen derived from the outer membrane of gram-negative bacteria. When introduced into the bloodstream, endotoxin can induce a febrile response, among other pyrogen-triggered systemic reactions, including septic shock in severe cases.

Any drug product or medical device that comes into contact with the bloodstream or cerebrospinal fluid is required to be tested for bacterial endotoxin following testing parameters outlined in United States Pharmacopeia (USP) Chapter <85>. Drug and product-specific monographs define the endotoxin limit required for their testing, meaning that the final product must have an endotoxin concentration lower than that defined limit.

Endotoxin testing is performed using Limulus Amebocyte Lysate (LAL) reagent, which is biological in nature and utilises a clotting mechanism found in the Atlantic Horseshoe Crab. Although the assay was initially established as a qualitative test, there are also various quantitative test methods that allow for the quantification of bacterial endotoxin in either a turbidimetric or colourimetric assay.

The assay, being biological in nature, establishes concentration in units of biological activity, or "Endotoxin Units (EU)", rather than in mass or size. At the molecular level, LPS naturally exists in various structures and formations, which can, in turn, affect the biological activity level of the molecule. Therefore, even if someone were to place two LPS molecules of the same mass side by side, their biological activity level may greatly differ.

Since endotoxin is a naturally occurring biological contaminant, it typically does not tend to pose a risk to patients under normal conditions with medications in the form of topical preparations, tablets, capsules and liquids. However, with injectable and implantable drugs and medical devices, these products that come into contact with the bloodstream or cerebrospinal spinal fluid can pose a major threat if contaminated with endotoxin.

Endotoxin limits for these products are set by accounting for the threshold pyrogenic dose (TPD), depending on the route of application. For intravenous injections, this dosage is 5 EU/kg, while for intrathecal (IT) applications, this dose is only 0.2 EU/kg. The TPD is the dosage of endotoxin that is capable of causing a fever, which was experimentally determined in rabbits with the precedent Rabbit Pyrogen Test (RPT). The endotoxin limit, typically reported in units of EU/mL, is then determined by dividing this threshold pyrogenic dose by the maximum bolus dose of the product in units of kg/hour.

Some products that should be tested for endotoxin include parenteral drug products, biopharmaceutical products (antibodies, recombinant proteins, cell and gene therapies), dialysis products and waters, injectable suspensions and emulsions, irrigation solutions, water for medical device manufacturing, immunotherapy products, intraocular devices, and surgical instruments and accessories, among various others.

Extractables and Leachables Testing: Background, Purpose and Testing Methodology

While bacterial endotoxin testing focuses on the detection of naturally occurring LPS from gram-negative bacteria, extractables and leachables testing has a more chemical and analytical technique. The primary purpose of E&L testing is to identify potential chemical migrants that may originate from drug product packaging, container closure systems, or manufacturing and bioprocessing components. The focus, rather than on a biological contaminant, is on chemical impurities in drug products and drug delivery systems that may cause long-term product instability or harm to the patient. These can also include formulation ingredients in the drug product, such as dyes, polymers, lubricants and antioxidants.

Unlike the use of a biological assay, extractables and leachables testing includes analytical and chemical analysis, including chromatography, spectroscopy, microextraction and total organic carbon (TOC) analysis. Typically, extractables risk assessments are performed on the container closure systems and packaging components, while leachables evaluations focus more on the drug products and stability testing of the final product, including its packaging and storage stability. While extractables testing is typically performed under exaggerated conditions, leachables testing involves the analysis of the product under normal storage conditions. Toxicological risk assessments are important to determine a patient's risk posed by chemical contaminants in a drug product to ensure that potential product instability is not a threat to patient safety.

The purpose of E&L testing is to identify the chemical contaminants and possible migrants from a drug product, drug delivery device, or critical manufacturing step. Products that need to be tested include combination drug and device products, parenteral drug products, biologics and biopharmaceuticals, tablets, topical applications, single-use

systems, manufacturing equipment, container closure systems, and implantable medical devices, among various others.

Overlap and Deviations Between the Assays

While there are significant differences between the processes and assays of E&L testing and endotoxin testing, there is also significant overlap in the products that are tested and the purpose of testing. While E&L testing is performed with the goal of identifying chemical and manufacturing contaminants, BET is performed with the goal of identifying a natural contaminant, LPS or bacterial endotoxin.

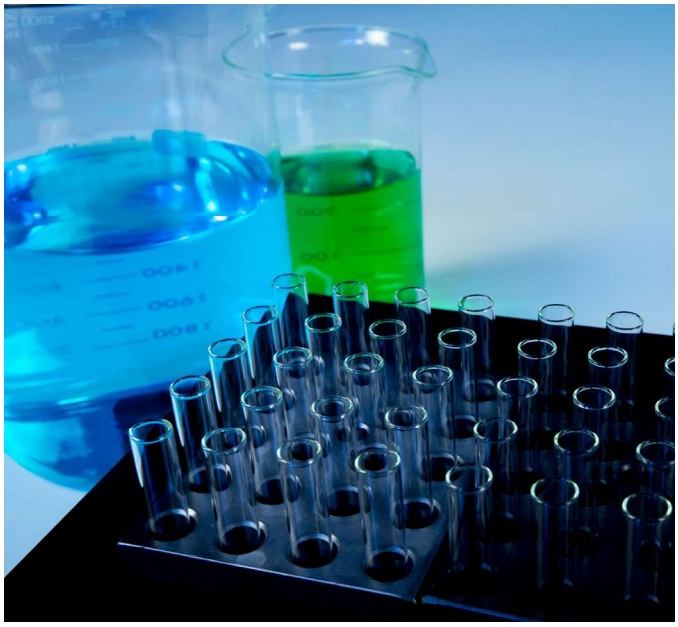
Parenteral drug products that are administered intravenously, intramuscularly or subcutaneously bypass the natural protective barriers of the body, increasing the risk of adverse reactions due to contamination in the product. Therefore, these injectable drug products require both endotoxin testing and extractables and leachables testing due to their contact with the bloodstream. Similarly, implantable medical devices that are in contact with sterile fluids, tissues, or bloodstream require both forms of testing as well. Examples of these types of products include catheters, hemodialysis membranes, implanted pumps and delivery systems, ophthalmic and intraocular devices, as well as surgical meshes and scaffolds.

Furthermore, combination products that integrate the process of drug delivery with a device component have significant overlap between the requirements for endotoxin testing and extractables and leachables testing. For these combination products, including pre-filled syringes and autoinjectors, BET and E&L testing are critical for both the drug portion as well as the device.

However, there are some products that require E&L testing that do not require BET. While E&L testing is required for oral tablet and topical medications, including ointments, eye drops and lotions, these products do not require BET because they are not injected or come in contact with the bloodstream or cerebrospinal fluid.

As mentioned, the methodology between the assays differs in origin, as E&L testing utilises chemical and analytical analyses, such as chromatography and mass spectrometry, while BET utilises the biologically based LAL reagent for endotoxin detection. Additionally, E&L testing often requires the development of risk-based assessments that include risk identification, analysis and evaluation steps for both the drug product as well as the packaging and container closure materials. Similarly, in this context, there would be the validation process and preliminary method suitability testing that is required for bacterial endotoxin testing.

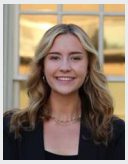
While the regulatory chapters that provide guidance for the testing methodologies differ, there is overlap in the regulatory bodies that provide oversight for these assays. The International Council for Harmonisation (ICH) provides harmonised guidelines for both extractables and leachables and bacterial endotoxin testing. ICH Q3E provides a comprehensive guideline for developing risk assessment and control for extractables and leachables, including chemical testing and assessment, as well as the evaluation criteria. Additionally, the USP provides guidance for informational purposes related to



E&L testing with above-1000 chapters <1661>, <1663> and <1664>. These chapters provide additional guidance on the assessment of extractables and leachables associated with drug products and packaging and delivery systems. General Chapters <661>, <232> and <233> also provide information on the assay procedures and chemical analysis for E&L testing.

Similarly, ICH Q4B Annex 14 provides harmonisation of guidelines from global pharmacopoeial bodies on bacterial endotoxin testing. This includes three major pharmacopeial chapters on BET: USP <85>, Japanese Pharmacopoeia (JP) 4.01 and European Pharmacopoeia (Ph. Eur.) 2.6.14. This also includes the assertion that all three reference standards for endotoxin testing (USP-RSE, JP-RSE and Ph. Eur. RSE) are interchangeable and can be used by LAL manufacturers for the development of secondary standards if needed.

While E&L testing and BET differ in their methodologies and analysis, they are both necessary assessments for ensuring patient safety through the identification of possible contaminants in drug products and medical devices. Many drug products and medical devices that require one of the tests may also require the other due to the overlap in the nature of the testing. However, each test serves a distinct purpose, as it is important to take into account all factors when considering patient safety.



Delaney Novak

Delaney Novak is a Technical Specialist for the Pyrogen Testing Division of FUJIFILM Biosciences. She holds a B.S. in Environmental Science alongside a minor in Biology. She enjoys working in a collaborative environment and is always open to addressing new challenges and answering complex questions. She applies these skills to further support any technical needs or concerns you may have in your pyrogen testing endeavours.