WP# 20 Bacterial Endotoxins - April 22, 2021

Background

Lipopolysaccharides (LPS), also known as endotoxins, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria. The term *lipooligosaccharide* ("LOS") is used to refer to a low-molecular-weight form of bacterial lipopolysaccharides. The toxic activity of LPS was first discovered and termed "endotoxin" by Richard Friedrich Johannes Pfeiffer, who distinguished between exotoxins (which he classified as a toxin that is released by bacteria into the surrounding environment) and endotoxins (which he considered to be a toxin kept "within" the bacterial cell and released only after destruction of the bacterial cell).

The potentially devastating effects of endotoxins to humans have been widely reported. In severe cases (at sufficient enough concentrations in the bloodstream) it is possible for endotoxins to cause irreversible side effects and even death. Bacterial endotoxins are found on the outer cell membrane of Gram-negative organisms and are pyrogenic (fever causing) by nature, as well as one of the most potent toxins known to man. Although these toxins are harmless outside of the body and in the alimentary canal, they are extremely potent once introduced into the bloodstream or intrathecally.

As a result of the dangers of bacterial endotoxins, the importance of testing for them is plainly apparent when looking at how susceptible and sensitive humans are to even minute traces. Introduction of these molecules can simply come from contact with contaminated parenteral devices or medications. It is particularly important to ensure that equipment and medication are free from endotoxins when caring for vulnerable patients. Bacterial endotoxins are ubiquitous in nature and are a menace for the manufacturers of all parenteral drugs and medical devices. Not only are they everywhere environmentally, they are very difficult to remove once they are introduced into a finished parenteral product.

In the early days of injectable pharmaceutical products, there was no method for testing for pyrogenic contaminants. It was the work of Florence Seibert and her team in the 1920s that brought forth the introduction of the Rabbit Pyrogen Test (RPT), and Frederick Bang / Jack Levin discovered the enzymatic pathway which caused the clotting of Limulus blood, and developed the Limulus Amebocyte Lysate (LAL test). During the 1970s, pharmaceutical companies experimented with the LAL test and found that it was a more cost effective, reliable and sensitive assay than the RPT.

Since its introduction, the LAL test has become one of the most important tools used in the pharmaceutical industry. It has allowed for a level of bacteria and pyrogen monitoring that simply eluded manufacturers when the RPT was the only pyrogen test available. The LAL test has ensured the absence of pyrogens in raw materials, water for injection systems, in-process samples, and in the final products.

Endotoxin Deactivation/Elimination

It is very difficult to deactivate and eliminate endotoxins. Endotoxin bacteria is extremely heat and pH stable; meaning it can endure through conditions most other parts of the cell may not. Endotoxin from a destroyed bacterial cell is elusive to say the least. The most ideal way to avoid endotoxin contamination is to prevent it from entering into your processes in the first place. Endotoxin is stable at high temperatures but it can be deactivated with heat. USP Chapter <797> recommends using a dry heat oven at 250 degrees Celsius for a minimum of 30 minutes to achieve sterility and depyrogenation.

Chlorine Dioxide (CD) Sterilization

Since endotoxins are created by the destruction of bacterial cell such as during a sterilization process, it is important to determine if excessive amounts of endotoxins are created when using CD to sterilize a medical device by killing the bacteria which are present. The following information is a case study of testing that was performed subsequent to a chlorine dioxide sterilization cycle.

Procedural Case Study

Subsequent to sterilization with gaseous chlorine dioxide, 3 lots of Medical Devices were examined for endotoxins. Each lot of samples was prepared and composite tested using 40 mL of pre-warmed 37°C LAL Reagent water per sample. The sample composites were extracted at room temperature for 1 hour with agitation on the mechanical shaker at 135 rpm. The extract was then tested per the Gel Clot Limits test using a lysate sensitivity of 0.03 EU/mL. After the completion of the Gel Clot Limits test the sample extract was tested for Inhibition/Enhancement per LSPEC #M407.

Efficacy Results

Bacterial Endotoxin - Gel Clot Limit Test			
Sample	**Tubes** 1 2 3 4	**EU/mL**	**EU/Device**
(3) [redacted] Lot #053-21	– – – –	**< 0.03**	**< 1.2**
(3) [redacted] Lot #054-21	– – – –	**< 0.04**	**< 1.2**
(3) [redacted] Lot #055-23	– – – –	**< 0.05**	**< 1.2**

(3) [redacted] Lot #053-21				
Product Positive Controls Series EU/mL	Replicate			
	1	2	3	4
2λ (0.06250)	+	+	+	+
λ (0.03125)	+	+	+	+
0.5λ (0.016)	-	-	-	-
0.25λ (0.008)	-	-	-	-

(3) [redacted] Lot #054-21				
Product Positive Controls Series EU/mL	Replicate			
	1	2	3	4
2λ (0.06250)	+	+	+	+
λ (0.03125)	+	+	+	+
0.5λ (0.016)	-	-	-	-
0.25λ (0.008)	-	-	-	-

(3) [redacted] Lot #055-21				
Product Positive Controls Series EU/mL	Replicate			
	1	2	3	4
2λ (0.06250)	+	+	+	+
λ (0.03125)	+	+	+	+
0.5λ (0.016)	-	-	-	-
0.25λ (0.008)	-	-	-	-

Standard Control Series - LRW		
LRW EU/mL	Replicate	
	1	2
2λ (0.06250)	+	+
λ (0.03125)	+	+
0.5λ (0.016)	-	-
0.25λ (0.008)	-	-

Summary

The (3) lots tested did not inhibit or enhance the LAL gel clot test. The sample composite lots tested were found to contain <0.03 EU/mL prior to performing the inhibition/enhancement test. The results of this test indicate there is no inhibition/enhancement occurring in the LAL gel clot assay.

In the sample composite extracts tested, <0.03 EU/mL, or <1.2 EU/Device was found. This result is well under the maximum allowable limit of 20.0 EU/Device for medical devices and under the significantly more stringent 2.15 EU/Device limit for cerebrospinal contact devices. This testing demonstrates that chlorine dioxide sterilization does not create endotoxin levels that exceed approved levels.