WP# 20 Bacterial Endotoxins - August 22, 2021

Background

Endotoxins are toxins present inside the outer membrane of Gram-negative bacteria that can be released when the cell disintegrates. They are also known as lipopolysaccharides (LPS) and consist of a lipid and a polysaccharide composed of O-antigen and an outer core and inner core joined by a covalent bond. 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 pyrogenic (fever causing) by nature, as well as one of the most potent toxins known to man. While harmless outside of the body and in the gastrointestinal tract, they are extremely potent once introduced into the bloodstream or intrathecally.

Introduction to bacterial endotoxins can come from contact with contaminated parenteral devices or medications, which considering their potential effect, illustrates the importance of testing these products prior to their sale and release. 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. In addition to being commonly found, 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. Since the 1970s, the Limulus Amebocyte Lysate (LAL) test has become one of the most important tools used in the pharmaceutical industry. Also known as the Bacterial Endotoxins Test (BET), the LAL test has ensured the absence of pyrogens in raw materials, water for injection systems, in-process samples, and in the final products. LAL tests can be performed both qualitatively to detect the presence of bacterial endotoxins, or quantitatively to determine the concentration of bacterial endotoxins present.

Endotoxin Deactivation/Elimination

It is very difficult to deactivate and eliminate endotoxins. The most ideal way to avoid endotoxin contamination is to prevent it from entering into your processes in the first place. Endotoxins can be deactivated with heat, with USP Chapter <797> recommending use of a dry heat oven at 250°C for a minimum of 30 minutes to achieve sterility and depyrogenation.

Chlorine Dioxide Gas Sterilization

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

Endotoxin Reduction Testing

A bacterial endotoxin challenge was conducted utilizing 304 and 316 SS coupons. A 4.1 log spike of E. coli endotoxin standard was placed on each set of coupons and then sterilization cycle using CD gas. The dosing parameters were 10,000ppm-hours dosage of ClO_2 at a 75% Rh. The 316 SS coupons had a 2.4 log reduction and the 304 SS coupons had a 4.1 log reduction.

Medical Device Case Study

Upon completion of a sterilization cycle using CD gas, 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 gas sterilization does not create endotoxin levels that exceed approved levels.