

## **What do you know about endotoxin?**

Cell culture contamination has been a major concern for researchers since cell culture was introduced in the early 1900's. One particular villain to the field of *in vitro* research has been endotoxin. But what do we really know about it?

### **What is Endotoxin?**

Endotoxin is a complex lipopolysaccharide (LPS), a toxic substance found in the outer membrane of most gram-negative bacteria. For example, one *E. coli* cell can contain up to 2 million LPS molecules. The bacteria releases the endotoxin into the culture environment while growing and when dying, possibly affecting the culture.

Studies of endotoxin *in vitro* have shown that they may affect the growth or performance of cultures and are a major source of experimental variation, although endotoxin does not affect all cells equally. Old cell lines that have been grown in culture for many years may have developed a resistance from long-term exposure before testing was common practice. CHO, 3T3, WI-38 and HeLa, for example.

### **Where does it come from and how do I stop it?**

**Sera** – Historically a major culprit for harboring endotoxin, but improved, automated screening has significantly reduced the risks. Many manufacturers offer Low-Endotoxin varieties of FBS, but this is not necessary for many cell lines.

**Water** - High-purity water is a must in any lab. The purification system should be well-maintained and storage conditions should be pristine. If possible, sterile water should be used promptly after filtration to avoid contamination during storage.

**Media and Supplements** – Finished media should be tested for endotoxin introduced by either the water used to dissolve the powder media or by the other media components.

**Glass and Plasticware** – Glassware, plastic tubing and fittings should be pyrogen-free, and reusables should be rinsed with pyrogen-free or low-endotoxin water. Plastic bottles should be sterilized by gamma irradiation.

**The User** – The proper aseptic technique while handling cell cultures, sera and media is necessary to minimize the risk of introducing endotoxin into a system.

### **How does Gemini Test for endotoxin?**

An *in vitro* test was developed in the 1970's which showed the Lysate of a horseshoe crab (*Limulus polyphemus*) to clot in the presence of small amounts of endotoxin. Gemini tests for endotoxin using the Limulus Amebocyte Lysate (LAL) kinetic chromogenic methodology that measures a color intensity that provides reliable, quantitative results, using a FDA-licensed test device that provides reliable, quantitative results, eliminating the possibility of subjective evaluation. This method of testing detects endotoxin in a range from 0.5 EU/mL down to as little as 0.005 EU/mL.

We look forward to bringing you more valuable cell culture information. If you have any questions, please contact your local Gemini representative.