Endotoxin Testing with a Contract Testing Laboratory

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Analytical Research Laboratories provides a variety of analytical and microbiological laboratory services: quality-control testing for nonsterile and sterile pharmaceutical compounds, biological testing, sterilization validation, consultation (troubleshooting to prevent contamination events, aseptic techniqueimprovement, small-scale compounding, etc.) and onsite evaluations. Endotoxin testing is one of our areas of expertise.

Endotoxins, which are components of the outer bacterial cell wall of gram-negative bacteria, are formed regardless of the pathogenicity of the bacterium when the integrity of the cell wall is lost. The endotoxin molecule consists primarily of cell-wall components as well as a polysaccharide and lipid A. Toxicity is associated with lipid A, and immunogenicity is associated with the polysaccharide component. Failure to perform pyrogen testing in a compounded drug can lead to the possible exposure of the patient to high levels of endotoxin, which can cause fever, diarrhea, septic shock, complement activation, and various nonspecific pathophysiologic signs and symptoms. As little as 5 EU/kg of endotoxin in a parenteral drug and 0.2 EU/kg in an intrathecal drug can cause a pyrogenic response. If an investigation after such an event reveals that proper endotoxin testing was not performed, the pharmacy could be closed and the owner subject to litigation.

Currently, the USP recognizes 2 endotoxin testing methods: the rabbit pyrogen test and the LAL method. The LAL method was developed after the discovery that amoeba-
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endotoxin screening. Potential sources of endotoxin contamination. Other sources of information on endotoxin testing are the Websites of the FDA (www.fda.gov/) and the Parenteral Drug Association (www.pda.org/). If a sterility failure occurs when a compound is tested and the offending organism is identified as Pseudomonas, the pharmacist should check the water source. An endotoxin test will likely reveal that there is endotoxin present in that water as well.

After receiving the results of an endotoxin test, the compounding pharmacist must evaluate whether the amount of endotoxin detected falls within acceptable limits, which are defined in the current edition of the USP, by the FDA, or (if an endotoxin limit is not provided in those sources) after calculation according to the following formula:

\[ \text{Endotoxin limit} = \frac{K}{M} \]

where \( K = 5 \text{ EU/kg for parenteral administration or 0.2 EU/kg for intrathecal administration} \) and \( M = \text{the maximum dose in milligrams per body weight or milliliters per body weight of the patient in kilograms per hour of the drug given.} \)

If the amount of endotoxin in the tested preparation is well below the established limit, then no further action is necessary. If the result is near or higher than that limit, then the pharmacist must decide whether to dispense or retest the compound.

Testing samples of raw materials for biologic contaminants and performing a valid endotoxin test on every high-risk-level sterile compound are critical components of good compounding practice—and quality compounding plus quality testing equals good medicine.