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 technique improvement, 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.[9] Toxicity is associated with lipid A, and immunogenicity is associated with the polysaccharide component.[10] 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.[11] 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 amebocytes in the blood of horseshoe crabs coagulate when exposed to endotoxin. The more endotoxin present, the faster the coagulation. When the LAL method was first developed, it was limited to the gel-clot method, in which multiple dilutions of a sample against a set of standards are used to detect the lowest dilution at which a reaction occurs or a gel forms. Two kinetic methods are also available for endotoxin detection: the turbidimetric assay and the chromogenic assay. Those assays require the use of a plate reader to detect a change in the turbidity or color of a tested sample, both of which occur if endotoxin is present. Kinetic methods permit the testing of multiple samples at a single dilution and thus produce faster results than does the gel-clot method. Multiple samples screened with a kinetic method can be analyzed simultaneously, and the concentration of endotoxin present is calculated automatically. However, with the LAL method of endotoxin testing, the following interferences can affect the results: beta-glucans (a false-positive result), a low or high pH (false negative), monovalent or divalent cations (false positive), endotoxin micelle formation (false negative), and a high concentration of sample (either false positive or false negative).

Endotoxin testing is not required for some sterile compounds (ophthalmic or otic preparations, creams, patches, etc.), but the types of preparations that should be screened for pyrogens include parenteral compounds of 25 or more identical items and intrathecal compounds (for which endotoxin limits are the most stringent), unless specified otherwise by the State Board of Pharmacy. All finished high-risk–level sterile compounds must be tested regardless of whether they have been poststerilized because most conventional sterilization performed with an autoclave, ethylene oxide, vaporized hydrogen peroxide, etc., does not remove or destroy endotoxin. The compounder should know the maximum dosage that will be prescribed by the clinician; that information can be used to establish limits for the prepara-

tion, especially if no monograph or guidance for the compound to be prepared exists. The difference in testing will be the maximum valid dilution that can be performed (a factor based on the endotoxin limit and the dosage and/or route of administration [i.e., IV vs intrathecal] of the compound).

The type of endotoxin testing most appropriate for an independent compounding pharmacy is often determined by whether the volume of the testing performed justifies the cost. When time is a factor and results are needed before a compound is administered, however, we suggest that the use of an in-house endotoxin test kit is preferable to submitting a sample to an independent laboratory for testing. If contract laboratory testing is deemed preferable, then determining the reliability and accuracy of the endotoxin testing offered is essential. The compounder should obtain information about the considered laboratory's history of pyrogen testing, the types of drugs tested, and whether that laboratory frequently tests the types of drugs contained in the samples that will be submitted. It is also important to determine whether the laboratory establishes data trends, so that (for example) if the result for a particular sample was always <0.1 EU/mg and then suddenly increased to 0.8 EU/mg, the laboratory analysts would immediately recognize those out-of-trend data and would check with the compounder about the source of that variation. An accurate and reliable endotoxin testing laboratory will explain the steps taken to handle out-of-specification samples and will ensure that interference properties did not cause the values of concern. The compounder should also confirm the accreditation of the laboratory considered and should ensure that the analysts there have passed a proficiency test in the performance of endotoxin screening.

The endotoxin limit in all raw materials used in compounding should be qualified and verified (by the vendor) to be within established limits; however, in some cases that task becomes the pharmacist's responsibility. Raw materials of botanical origin should always be screened for endotoxin, but many raw materials have inherent properties that are endotoxin-like. In those cases, other analyses (such as aflatoxin testing) may be necessary.

The current USP Chapters <85> and <797> clarify testing requirements and compounding best practices for endotoxin screening, and Chapters 51, 61, 62, 71, 1116, and 1111 are focused on microbial control. The tests described in the USP will help to identify 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:

Endotoxin limit = K/M

where K = 5 EU/kg for parenteral administration or 0.2 EU/kg for intrathecal administration and M = 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.