Allowable Endotoxin Limits

Bacterial endotoxins, including what they are, how to minimize them and their acceptable limits in compounded preparations, may be one of the least understood areas of microbiology. To adequately address all the subtleties of the subject could easily take much more space than I have in this column; however, a short review may help clear up several of the more important questions.

What Are Bacterial Endotoxins?
Bacterial endotoxins are sometimes referred to as pyrogens. In fact, bacterial endotoxins are only one form of the broad class of “microbial pyrogens.” Fortunately, these bacterial endotoxins are the primary type of pyrogens that are most significant to the pharmaceutical industry. Most pharmacists refer to “pyrogens” and “endotoxins” as the same thing and these terms have come to mean the same – bacterial endotoxins.

Bacterial endotoxins, found in the outer membrane of gram-negative bacteria, are members of a class of phospholipids called lipopolysaccharides (LPS). LPS are not exogenous products of gram negative bacteria. The release of LPS from bacteria takes place after death and lysis of the cell. Good examples of endotoxin producing gram-negative bacteria are *Escherichia coli*, *Proteus*, *Pseudomonas*, *Enterobacter* and *Klebsiella*.

How Do I Minimize Endotoxins In My Preparations?
The key to minimizing endotoxins is to remember where they originate – from dead gram-negative bacteria. For example, steam sterilizing glassware that has been rinsed with tap water, while eliminating active microbes, may actually increase the endotoxins on the glassware. The best way to eliminate endotoxins from glassware is to heat to 250°C for two hours. This also is the reason that you want to sterilize your preparation as soon as possible. Time allows bacteria to multiply in a non-sterile preparation. Heat or steam sterilization will kill the bacteria, but you may be left with excessive amounts of endotoxins.

Passing a preparation through a 0.22 µ filter, while removing bacterial and fungal contamination, does not remove endotoxins. Some positively charged filters can lower the endotoxin levels, but the action is electrostatic rather than a sieving mechanism, so complete removal is usually not possible.

In general there are three areas that can help keep endotoxins under the allowable limit:

- Use endotoxin-free glassware and implements in your compounding;
- Use sterile water that has been tested to have less than 0.25 Endotoxin Units/ml, and;
- Use chemicals from a reputable source.

What Is The Endotoxin Limit For My Preparation?
Fortunately, the human body can tolerate small amounts of bacterial endotoxins. Specifically, if administered via IV, the level is 5 EU/kg-hr; and if administered via IT, the level is 0.2 EU/kg-hr (where EU = Endotoxin Units; kg = body weight in kilograms). So, if you know: (1) the patient weight; (2) the route of administration; and (3) the amount of the preparation to be administered in one hour, in milliliters, you can calculate the EU/mL limit. Most calculations assume a person weighs 70 kg, but this may need to be adjusted, especially for children. Where the amount to be administered is not known, it is assumed that the maximum dosage level is given.

So, if you have an IV preparation of 5 mL that is being given all at once to a child weighing 25 kg, the endotoxin limit is:

$$\text{EU/mL} = \frac{5 \text{ EU/kg-hr} \times 25 \text{ kg}}{5 \text{ mL}} = 25 \text{ EU/mL}$$

When we test for the endotoxin content of a preparation we assume a body weight of 70 kg, and ask for the administration route and the dosage in mL/hr. Using this information we can determine if the preparation passes or fails the test limits for bacterial endotoxins. There are several special cases for endotoxin limits, the most important being sterile water for injection, which must be below 0.25 EU/mL, and bacteriostatic water for injection and sterile water for inhalation, which must be under 0.5 EU/mL.

Testing your compounded sterile preparations for bacterial endotoxins is an exacting process requiring specialized equipment, chemicals and trained personnel. Many in-pharmacy tests are difficult to perform and subject to errors in operator technique. USP <797> requires endotoxin testing for many sterile preparations, so it may be a good idea to have a contract laboratory perform the tests so that you will have confidence in the endotoxin levels.

For more information, please call Eagle Analytical Services at 800/745-8916.