USP Chapter <85> - FAQs

What is the USP <85> Test?

The Bacterial Endotoxins Test (BET) is a test to detect or quantify endotoxins from Gram-negative bacteria using amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). The result will indicated if the preparation tested is below or exceeds an endotoxin limit.

When is a USP <85> Test Required?

USP <797> states: All high-risk level CSPs, except those for inhalation and ophthalmic administration, prepared in groups of more than 25 identical individual single-dose packages or in MDVs for administration to multiple patients or that are exposed longer than 12 hours at 2° to 8° (C) and longer than 6 hours at warmer than 8° (C) before they are sterilized shall be tested to ensure that they do not contain excessive bacterial endotoxins. Some accreditations boards recommend frequently performing USP <85> testing on lots less than 25 identical packages. Check with your individual state regulations.

How are Endotoxin Limits Calculated?

Endotoxin limits are determined by the maximum bolus of a product per kg of body weight, or maximum total dose administered in a single hour, if administered at frequent intervals or infused continuously. They can be expressed as mL per EU (endotoxin units) or mg per EU.

What Methods are Used?

1. Gel-clot technique – based on gel formation
2. Turbidimetric technique - based on the development of turbidity after cleavage of an endogenous substrate. This technique is a photometric assay measuring increases in reactant turbidity.
3. Chromagenic technique – based on the development of color after cleavage of a synthetic peptide-chromagen complex.

Which Method is Best for my Formula?

Your testing laboratory will determine the most appropriate method of testing according to the formulation, the concentration of ingredients, any potential interference with the methods, past experience and SOPs.

How is the Result Reported?

They are reported as a concentration of EU/mL or EU/mg versus the endotoxin limit. If the result is lower than the limit it passes, if the result exceeds the limit it fails.

Can I Perform my Own USP <85>

Kits and bench top testing units are available. It is important to ensure that these systems are appropriately calibrated, validated and maintained to ensure accurate results. A database of endotoxin limits for each formulation should be maintained with calculations for reference. Some testing systems are available with tracking software.

Sources: USP Chapters <797>, <85> Current with USP 38-NF 33