Potential Alternate Bacterial Endotoxins Testing (BET) based on chemical analytical methods – Opportunities for Industry:

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Bacterial Endotoxins in the pharmaceutical products are currently being tested using USP method <85> (1) which utilizes LAL (Limulus Amoebocyte Lysate) reagent. The LAL reagent formulated from blood cells of horseshoe crabs. As LAL assay relies on the blood of wild horseshoe crabs, the conservation of global horseshoe crab population has become a potential issue. Due to this reason and not to depend solely on reagents derived from animal origin, several synthetic variants of the LAL reagent, recombinant factor C (rFC) and recombinant cascade reagents (rCR's), have been developed by several vendors and they are actively pursuing to make the rFC and rCR's as an alternate reagent to LAL for endotoxin assay. However, based on the published literature, USP believes still much work is needed to establish these recombinant reagents' comparability to the LAL in Endotoxins Test, as indicated in USP<85>.

There are few reports of chemical methods of endotoxin analysis using GC-MS (2,3)) and LC-MS (3-10) for the occupational hazard testing purpose. Some of these methods measure 3-hydroxy fatty acids in Lipid A, after hydrolysis of lipopolysaccharide. The advantage of these methods includes estimating the total amount of endotoxins (cell-bound and non-cell bound) present in the sample and also not dependent on the inhibition and/or activation of the enzymatic reaction due to substances of non-bacterial origin (beta-glucans) or the presence of peptidoglycans. A good correlation was shown when these chemical analysis results compared to the LAL test (9).

Since an Endotoxin is a lipopolysaccharide (Lipid A + Polysaccharide), chemical analysis methods can potentially be developed as alternate methods for bacterial endotoxins testing. Methods can be developed for either analysis of the component of Lipid A (3-hydroxy fatty acids) using GC-MS or LC-MS or carbohydrate analysis of the Polysaccharide using carbohydrate analysis (11).

Our goal is to engage the pharma industry and regulatory agencies for their perspective with respective to such an approach. The questions are:

- Why there is no progress has been made in the chemical analysis methods as they have become routine, easy to use, and can be automated to run hundreds of samples per day (high throughput)?
- What are the main roadblocks for developing and implementing these methods?
- What are some of the regulatory issues if the industry is willing to implement them?

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