

FDA and USP can help promote horseshoe crab conservation

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Limulus polyphemus

To help assure product quality and safety, pharmaceutical, biopharmaceutical and medical device manufacturers test products such as injectable drugs, vaccines, large volume parenterals, and implantable devices for endotoxin content in addition to sterility. The most prevalent method for testing for endotoxin is a test derived from the blood cells of horseshoe crabs. In the United States, the Atlantic horseshoe crab, *Limulus polyphemus*, is the source of LAL. One of the three species of horseshoe crabs indigenous to Asia, *Tachypleus tridentatus*, is used primarily to make TAL. LAL is used globally; TAL is typically used in Asia.

In the US, the Food and Drug Administration (FDA) regulates the manufacture of pharmaceuticals and biological therapeutics such as insulin injections, flu vaccines, etc., and medical devices such as knee replacements, and stents. In 1987, with an amendment in 1991, the FDA issued guidance to industry regarding how to use the LAL test for final release of the products that fell in its jurisdiction. The US Pharmacopeia (USP) publishes a referee test for endotoxin, Chapter <85> Bacterial Endotoxins Test (BET)¹. The intent of a referee test is to provide the method that should be used when there is a dispute between test results. Although many of the requirements in the BET are similar to what was included in the FDA LAL guidance document, the FDA document provided information and recommendations for topics not included in the BET chapter. The two documents complemented each other, but one does not completely replace the other.

FDA LAL guidance document obsolete

On 12 July 2011, the FDA withdrew the LAL guidance document. The announcement received by the LAL manufacturers included a statement

indicating that the USP BET provides information on the performance and acceptance criteria for endotoxin testing. The FDA stated the LAL guidance document no longer reflects its current opinions.

Recently Sutton and Tirumalai² described the work completed by the USP Microbiology and Sterility Assurance Expert Committee during the 2005 to 2010 revision cycle. The article included changes and publications reviewed and published during the cycle. In the article, the authors reiterated that USP chapters are not intended to be used for batch release.

With no new guidance from the FDA and a USP chapter that is not intended for final product release, and therefore does not include recommendations on things such as the use of control standard endotoxin (CSE), archived curves and how to determine the appropriate positive product control (PPC) concentration, end-users of the LAL test are left with a gap. The LAL vendors felt compelled to provide guidance to their customers knowing that their opinions may not be those of the FDA inspectors. It is now critical that the BET be revised to address the items that were included in the FDA LAL guidance document for which the current BET is silent.

USP seems to be aware of a recombinant alternative to LAL

Sutton and Tirumalai² briefly stated the need to update the BET to accommodate new technologies for endotoxin detection, including the recombinant Factor C (rFC) method used by Thorne et al.³ The authors did not mention that the expert committee reviewed and an article for stimuli was published in 2010 that addressed a comparison of an alternative rFC method and LAL⁴. The rFC method in the article for stimuli is the method used by Thorne et al.³ and was commercialized by Lonza. The PyroGene™ rFC assay uses a recombinant form of the same proenzyme Factor C, that is present in LAL and TAL, manufactured using a cell culture method. Factor C is involved in the initial binding of endotoxin and its activation is the starting point for all assay methods for the detection and measurement of endotoxin. LAL and TAL methods use gelation, turbidity or color change to detect the activation of Factor C and therefore, the presence of endotoxin. The rFC method uses fluorescence.

Other companies claim to have rFC assays commercially available. We challenge those companies to validate their alternative rFC method similarly, according to USP Chapter <1225>⁵, and submit their data as an article for stimuli. This can help further stimulate the discussion of alternative methods, their applicability for endotoxin detection, and the appropriateness of being included as photometric methods in the BET.

Why do we need an LAL and TAL alternative?

Chapter <1225> requires the statement of a rationale section for validating an alternative be included to identify the need for the alternate procedure. During the pre-publication review process, the USP committee requested that this section be removed from the Lonza rFC article for stimuli. Our rationale for the development of the rFC assay was to provide the pharmaceutical, biopharma and medical device industries with a comparable yet sustainable test that does not require the use of animals in its manufacture.

Although the LAL and TAL methods have all but replaced an animal-based test called the rabbit pyrogen test for endotoxin detection, they still require that an animal be used in the manufacture of the LAL and TAL products. Horseshoe crabs are collected from beaches, shallow water and deep ocean water and brought to bleeding facilities. In the United States, a portion of their blood is removed and many of the crabs are returned to the waters from which they were removed. Some LAL companies give the horseshoe crabs to bait fisherman after they are bled. All horseshoe crabs bled by Lonza are returned to the ocean. Horseshoe crab handling methods for TAL manufacturers are not known as well as the US methods, but it is believed most crabs die post-bleeding, some as bait, some as food, and some due to the bleeding process itself. In June, representatives from US-based LAL vendors presented best practices for harvesting and handling methods at the International Workshop on Science and Conservation of Asian Horseshoe Crabs to help change local practices that are affecting horseshoe crab populations in Asia.

Without change, LAL and TAL are not sustainable

The global demand for LAL and TAL increases annually as the demand for drugs and devices increases. The Asian population of horseshoe crabs is in decline and in some areas, horseshoe crabs can no longer be found. A steady decline in the Asian horseshoe crab population means a decline in the basic raw material needed to make TAL. Less TAL means more of a demand for LAL for the Asian market. In the US, harvest restrictions for the LAL vendors are being considered which would limit the amount of LAL that can be manufactured.

Until the FDA or the USP openly allows for the use of alternative recombinant methods, those who are required to use the BET test for release of their products to market appear hesitant to move away from the animal-based test and implement the recombinant alternative. This puts all of us who need products such as intravenous solutions, injectable drugs, vaccines, and implantable devices at risk. The FDA has the option to include the acceptability of a comparable recombinant assay in a replacement endotoxin test guidance document. As Sutton and Tirumalai² suggest, the USP can open up the BET chapter for revision and include as a photometric method the rFC method already shown to be comparable to a current compendial method⁴. This will open the door for other vendors to prove their recombinant methods, too, are equivalent. As the USP committee responsible for revisions to the BET chapter strives to harmonize

the chapter so that it is consistent between the US, Europe and Japan, including an alternative recombinant assay can affect the demand for both LAL and TAL.

Recombinant alternative can help preserve horseshoe crabs

Acceptance of a recombinant alternative by the FDA and the USP and the implementation by pharmaceutical, biopharmaceutical and medical device companies allows for a reduction in the use of a natural resource, the horseshoe crab. In addition to mitigating the risk to the availability of products needed for public health, such as vaccines, the use of the recombinant alternative promotes the conservation of this extraordinary species.

References

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