

Table 16: Endotoxin Testing

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SCOPE:

Ensuring the safety of medicines is a priority for both manufacturers and regulators. For parenteral products, this concern for safety requires that the preparation of the drug product meet, among other tests, "... Pharmacopeial requirements for sterility, pyrogens,..." (USP<1>). Drug products claiming to be sterile and pyrogen-free are also required to comply with 21 CFR 211.167(a). Biologics need to meet additional requirements outlined in 21 CFR 601.2(d) and 21 CFR 610.13(b).

The various Compendia, (USP<85>, Ph. Eur. 2.6.14 and JP 4.01), provide validated methods for the sensitive detection and quantitation of bacterial endotoxin. These Compendial methods have proven to ensure product safety but with ever-increasing complexity of drug product formulations comes the challenge of ensuring that excipients, and combinations thereof, do not pose a barrier for the reliable and sensitive detection of bacterial endotoxin. Chen et al (2013) reported on the phenomenon of low endotoxin recovery (LER), ushering in a greater scrutiny of the LAL test methods for biologics, especially those with certain formulations.

The goal of this roundtable is to explore strategies and best practices employed for endotoxin testing to support biologics development.

QUESTIONS FOR DISCUSSION:

1. At what time points, and for which conditions is endotoxin testing incorporated in stability studies? If not included in stability studies, what risk mitigation is used to justify exclusion?
2. To what extent are your formulation development decisions and manufacturing operations informed by LER concerns? Is it matter of establishing product stability first and worrying about LER later?
3. At what point in the development cycle does LER come into focus?
4. What method(s), excipients and surfactants have been used to successfully overcome LER for your product(s)?
5. What challenges arise when implementing an alternative LAL test method at a preferred CRO not using the required reagents? How have these challenges been overcome?

DISCUSSION NOTES:

8 participants from 6 different companies were represented at the table discussion. Two particular resources were identified for endotoxin testing and LER issues. The PDA technical report #82 was identified as a comprehensive resource regarding endotoxin testing, including issues associated with LER and regulatory expectations regarding such studies. Another good resource identified was the book "Endotoxin Detection and Control in Pharma, Limulus, and Mammalian Systems" edited by Kevin Williams.

1. Regarding endotoxin testing in stability protocols:

- a. One company had ET only at DP release, and was asked by health authorities to include it yearly. It has now part of routine stability studies, starting in phase 1 (either yearly, or at the start and end of stability studies)
 - b. Another company has success performing ET only at release and end of shelf-life, but ROW countries with shorter shelf life can pose an issue (if ET wasn't tested at that timepoint)
 - c. A 3rd company also has end of shelf-life ET testing as part of stability protocols, but is starting to incorporate the testing in stability studies at earlier timepoints in studies following process lock.
 - d. Companies have had mixed success using CCI testing as a surrogate assay to support not having endotoxin testing on stability protocols. Most are performing ET testing at release and end of shelf-life.
2. Scrutiny of LER continues to be primarily driven by CDER, and companies have not seen similar levels scrutiny from other regulatory authorities or divisions within FDA. One company is trying to perform LER studies earlier in development to mitigate future risks.
 3. All companies continue to use CSE after calibration against RSE for routine testing.
 4. No company indicated that formulation or process development is driven by a desire to avoid LER issues. With respect to formulation selection, avoiding LER would not be a primary selection criteria. One company expressed an opinion that the product has an influence on LER in addition to formulation, and that LER is not predictable based on formulation alone. This company cited experience with multiple products in the same formulation some of which did and some of which did not have LER.
 5. The earliest point at which LER assessment can be done is after commercial DP process and formulation lock, in particular definition of hold times. LER evaluation needs to be done reflecting both the process conditions (temperature, hold time, etc) and analytical conditions (temperature, sample hold times, etc).
 - a. Multiple companies evaluate LER after a soft lock, or at some other point prior to process validation. Earlier LER evaluation doesn't eliminate the need to assess LER as part of validation, but can trigger analytical development work.
 - b. Remediation of LER issues can be technically difficult and potentially time consuming. PDA report #82 includes a chapter regarding potential approaches to address LER.
 - c. A worst-case outcome of LER could be adjustment of process hold times in order to avoid unacceptable LER.
 6. One company has found the vial types used for standard and sample preps has a substantial influence on LER, particularly at 25°C. As an example, round bottom vials were found to offer superior performance to other geometries. Exact types of glass were also discussed as a possible variable in LER. Method optimizations conducted previously at colder temperatures may not hold for testing at 25°C. Sample mixing time can also be a parameter to consider.
 7. Alternatives to LAL testing were discussed:
 - a. One company is developing cell-based monocyte activation assays for endotoxin as an alternate method for endotoxin testing that may be less susceptible to LER, including development of in-house cell lines.
 - b. Recombinant factor C assay testing is an increasingly popular alternative to LAL assays, but requires full validation and is not currently a USP method. LER issues have also been observed with the recombinant factor C assay. One company expressed the perspective that simpler critical reagent qualification may be an advantage of the recombinant factor C assays. The assay also has better specificity (eg, no glucan cross-reactivity) compared to LAL methods. The method may be included in USP soon.
 - c. MAT (monocyte activation testing) is being evaluated by some as a back-up method over rabbit pyrogen testing in the situation where LER issues cannot be resolved. Multiple companies are performing MAT method development in house. These assays were

widely recognized to be challenging, and it may be preferable to utilize CROs. In general, LER by BET is also seen in MAT so it may not offer advantages. Multiple companies are working with Microcat Biotechnologie to establish platform MAT methods.