Table 12: Low Endotoxin Recovery (LER)

Facilitators –

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Scope:

LER, a phenomenon discovered in 2013, was reported as time-and-temperature-dependent masking of endotoxins spiked into biological samples, resulting in failure of endotoxins detection by compendial Limulus amebocyte lysate (LAL) method. LER issue poses safety concerns for sterile products as un-detected endotoxins in contaminated products can be pyrogenic. FDA and vendors have collaborated over the past years, investigating the root-cause and identifying risk-mitigation strategies.

In an effort to provide guidance and harmonize LER studies, PDA published a technical report TR No. 82 in 2019, primarily for protein drug products. The TR recommends that 1) LER hold-time studies be conducted using the validated endotoxin test method used for routine testing; 2) LER studies be performed on three product batches and conducted under process relevant conditions. Currently it is expected to present LER hold-time studies in BLA. If LER is detected, additional tests (usually Rabbit Pyrogen Testing) are required for batch release until the masking effects is overcome.

This roundtable's discussion will explore best practices for detecting and overcoming the LER issue.

Questions for Discussion:

1. At what development stage is LER study initiated?
2. Are any manufacturing process or formulation development decisions driven by LER considerations?
3. What method(s), excipients and surfactants has been used to successfully overcome LER?
4. Is LER hold-time data or alternative data provided in BLA/MAA? Were LER questions received from health authorities other than FDA?
5. What are the key parameters (eg hold times) considered in developing the protocol for LER hold time study? Is the BPOG protocol acceptable to regulatory authorities?

6. How is endotoxin recovery calculated in LER study? Using the method recommend by PDA TR82?
7. Is endotoxin test performed on DS stability? Was this driven by Health authority request?
8. Are there alternative testing methods to LAL to overcome LER effect?

Discussion Notes:

February 2 –

- It is recommended to start LER study as early as possible, at least a year before PPQ batches. Because it is difficult to predict LER and takes time to overcome the LER. Eg. Four different strengths of a drug formulation, conducted LER in all four strengths. Most pronounced issue is in low concentration. Surprising but indicated need to do the study on formulations/strengths being considered. So far FDA CDER is the only one asking for this data.

- No, platform knowledge is not considered beneficial. Product to product LER evaluation is needed as it is product dependent. 12 products were evaluated in same base formulation, exhibited different levels of LER. Could be that the pockets of charges on the protein molecule in hydrophobic regions, location of which could vary depending on environment and hence the impact on Endotoxin recovery and LER varies – current hypothesis. So, platform information not applicable

- Chelators to be avoided in formulation eg EDTA

- Endotoxin is performed as release testing mainly, but some HAs like FDA like to have data at EOS, hence testing done on stability as well.

- Data mainly needed for registration and inspection. FDA CDER requires LER data, so far, no experience with CBER asking for this data

- LER issue even without Polysorbate 80/20 in the formulation matrix observed and so very difficult to predict LER. Initially it was thought to be citrate and Polysorbate. Over time, realized many more chelators (any chelator, not just citrate), with PS, causes LER. Also, sometimes addition of protein lessened the LER effect…very unpredictable. TR82 tells how to distinguish between effect of different excipients in formulation on LER

- CRO Company in Germany, Microcoat Biotechnologie has been used to develop demasking assays. …Key contact is Johannes Reich (also author on TR82)

- Hold time study: Steps in manufacturing process where contamination most likely to occur and processing conditions determine study design, recommend looking at TR82, that recommends worst case temperature. Use a reverse protocol, make sure there are additional timepoints

- TR82 trumps BPOG paper since regulators do not necessarily agree with the BPOG protocol, especially use of NOE, not accepted

- Methods alternative to LAL: Recombinant factor c and different LAL reagent sources, case studies in TR82. Unpredictable. MAT test has been shown promising, however requires an extensive amount of work to develop a robust MAT method. MAT can be considered a good alternative to LAL and RPT…lot of work to do though, need expertise in MAT, EP 2630 useful as a general guideline, but hard to implement. A successfully developed MAT may be deemed acceptable by FDA and EMA as an alternative to LAL test shown to have LER issue, thereby not requiring ongoing RPT on release post approval

- No questions from EMA and other HAs

- No expectations of approved product to comply with TR81 or generate LER data

- Data driven. If you reach close to submission, you run RPT, until LER issue is resolved, usually as a PMC. Very rarely, controls strategy accepted, mostly is to do RPT until LER issue is resolved

- What type of endotoxin standard is more representative and should be used for the LER study? The recommendation from TR82 and FDA is to use well-characterized endotoxin standard RSE/CSE provided by LAL vendors. NOE not accepted at all by FDA

- Out of 12-15 Mab products evaluated for LER, Strong LER observed in 10-20%, and assay variability in 10-20%, and 60-80% had no issues at all relating to LAL test