Roundtable Session 2 – Table 8 – Best Practices for Execution of Low Endotoxin Recovery (LER) Studies

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Abstract:

The pharmaceutical industry recently encountered a significant challenge called Low Endotoxin Recovery (LER) in their pharmaceutical products. Companies have developed various strategies to understand LER and prove their products pose no safety risks. This roundtable discussion focuses on sharing industry best practices and experiences in managing LER challenges.

Discussion topics:

(1) Experience with LER issues in your products

(2) Risk mitigation strategies and solutions implemented

(3) Regulatory agency feedback and specific questions encountered

(4) Industry best practices for addressing LER challenges

Notes

1. **Why LER Became a Focus Area**

LER was originally reported in 2013, as a phenomenon where low recovery of endotoxin was observed when endotoxin standard is spiked into some formulated drugs. However, no real-world patient harm has been reported. However, LER remains controversial, due to the nature of endotoxin standard being spiked for the study as well as lack of reported patient-impact cases from LER. Rabbit pyrogen testing, though less sensitive/variable, is still requested, creating inconsistency in expectations across regulatory agencies.

2. **General Industry Experience**

Participants represented biosimilar manufacturers, small companies, and Regulatory CMC teams. LER has been discussed since around 2013, and some consider the requirement scientifically unnecessary, while others believe it is important to fully evaluate endotoxin-masking risks.

Companies have in the past described products where extensive method development could not resolve LER issues; MAT was unsuccessful, leaving rabbit pyrogen testing as the only option, but this method is not accepted by EMA. Specialized contract labs could not overcome

masking, even though routine release testing remained compliant. This leaves some companies in a very tricky place for their filings.

Although companies often prefer MAT, agencies frequently insist on pyrogen testing, triggering comparability requirements. For example, it has been the experience of several attendees that some agencies often request rabbit pyrogen testing when LER cannot be resolved through traditional methods, but some health agencies are comparatively flexible and may accept LER data generated under routine conditions without additional rabbit pyrogen testing, if justified scientifically for how your process removes and controls endotoxins.

Some companies have been successful in pushing back on LER related requests using strong technical rationale. Compressed regulatory timelines make it difficult for companies to prepare and justify with additional data packages.

3. Product-specific issues

Products containing polysorbate and chelating agents (e.g., EDTA) were highlighted as particularly vulnerable to LER study failures. One company noted agency feedback regarding the lack of LER sensitivity of specific CSE lot. Companies noted the value of direct communication with vendors to understand differences between CSEr lots, if any, and how we should use their broad expertise to ensure this area of a filing carries minimal risk.

4. Issues with qualified standards

RSE and CSE standards are highly purified, lyophilised endotoxin preparations that may behave differently than the natural endotoxin that may be present in manufacturing processes but remain as regulatory expectation. These are made and controlled by well-known manufacturers , but an example was shared of an agency deficiency letter which questioned the endotoxin standard particularly that the study did not represent true worst-case conditions. The company ultimately had to demonstrate that their validated method worked only with that specific standard, and also to commit to performing additional rabbit-pyrogen method studies.

5. Study Design Best Practices

Temperature control and reducing assay variability are critical; reverse-spiking (Spiking samples based on time interval and testing all samples at the end) was highlighted as helpful. Hold-time considerations were discussed; while the study conditions are not typically based on manufacturing hold times unless open or higher-risk steps exist, some companies incorporate sample transit time to QC and typical testing turnaround into study design. The study design should also be tailored to the specific product (DS/DP).

The method chosen for studies also matters; chromogenic vs turbidimetric LAL methods may show different levels of sensitivity to interference. If possible, performing your LER studies earlier in development is beneficial (when formulation is fixed already), as it may help to de-risk a program. Performing your study on 3 x PPQ batches is still the common practice for submissions.

Platform data may be leveraged when formulations are similar to justify LER related issues in the regulatory submission. High-concentration formulations can complicate platform applicability. Even if not accepted as the primary justification, platform data remains valuable supporting evidence.

6. When LER Cannot Be Resolved

If dilution, reagents, or rabbit pyrogen methods cannot mitigate the issue, companies rely on a rationale-based approach using the following; demonstrating robust endotoxin clearance throughout the process, showing consistent historical batch data, confirming that the manufacturing process is closed and low-risk, and demonstrating that patient exposure is minimal and clinically safe.