Regulatory Challenges of Recent Low Endotoxin Recovery Queries

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June 25, 2026

Agenda

1

Background and Expectations

- Parenteral Drug Association Technical Report 82 Guidance

2

Summarize most common queries

3

Share select examples of queries that have required changes to Pfizer's LER strategy

4

Misalignment of Queries from Guidance and Impact

Low Endotoxin Recovery (LER) ≠ BET ≠ Endotoxin Sample Hold Time study!!

Technical Report No. 82

Low Endotoxin Recovery

1.0 Introduction

The term "low endotoxin recovery" (LER) describes the failure to detect spiked endotoxin in some finished sterile biological drug products when tested using the internationally harmonized compendial *Limulus* amebocyte lysate (LAL) assay to detect bacterial endotoxin *(1–6)*. Regulators and developers became concerned that compendial methods required by international regulations were unable to confirm the absence of endotoxin in certain drug products, especially troubling considering the inherent risks pose

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cal data provide no direct evidence of LAL failing to detect pyrogenic products.

In 2013, the U.S. FDA began to request applicants submitting biologics licensing applications (BLA) to provide evidence that endotoxin spiked into a drug product could be detected using the USP <85> BET. Referring to confidential data from spiking studies submitted in BLAs, the FDA reported in 2014 and 2015 that poor endotoxin recoveries were observed in some cases, which confirmed the initial findings of Chen and Vinther. *(1,8,9)* The data from endotoxin spike/hold studies presented in BLAs

Low Endotoxin Recovery (LER)
The inability to recover ≥50% activity over time when known amount of endotoxin is added to an undiluted product. LER cannot be overcome by dilution.

What is it (LER), exactly?

Low Endotoxin Recovery – a concern emerging in ~2015 from FDA CDER micro BLA reviewers that the traditional BET may not detect “masked” endotoxin

A one-time *research study* where *drug products* are spiked with endotoxin and recovery measured over process-relevant hold times

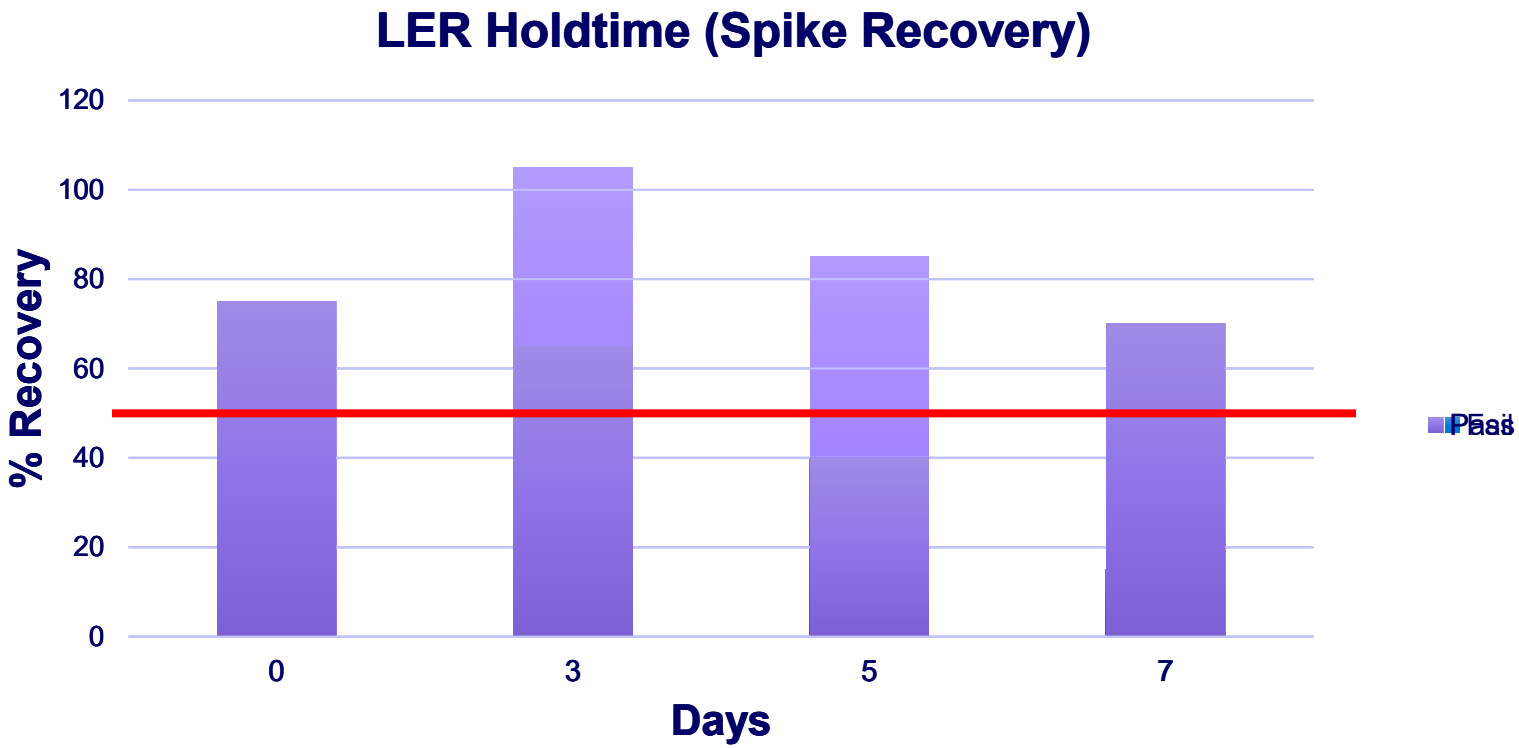
Peer reviewed publication in March 2019 (PDA TR82)

EMA added LER to Q&A for Biological Medicinal Products in September 2023

Currently filed in BLA (CDER only) and MAA

How do we know if the assay has LER?

An endotoxin spiking study on a final drug product sample with a minimum of four time-points, is tested for recovery over a course of time (based on DP process parameters). Two consecutive results below 50% recovery fails (has LER).



Low Endotoxin Recovery (LER) Query Themes

Queries from multiple countries

Queries to submit entire LER report

Additional details are required

- Nominal CSE spike concentration in water control
- BET method used in LER study
- Vendor and part number of CSE used

Additional queries outside parameters of TR82

- In-process and DS samples
- Manufacturing through lab sample hold time
- One non-consecutive time point below 50% recovery is failing LER

Queries – Multiple Countries 2023, 2024, and 2025

Example Queries

- Please submit the result of LER (Low endotoxin recovery) for drug product – Korea
- In the low endotoxin recovery study, explain the noted decline in the results by day 3 and their increase on the following time point (4th day) – Saudi Arabia

Countries other than U.S. and EU do not have guidance or definition of LER

- Countries outside of US and EU requesting LER information
- Only agency included in authoring of TR82 - U.S. FDA
- EMA Q&A now points to TR82 as guidance
- Difficult to anticipate when LER study is expected from agencies other than FDA and EMA

Query # 1 – EMA Query – 2021

Since pre-treatment of samples is performed only on DP samples, the Applicant should provide summary of studies performed for low endotoxin recovery (LER) on DP samples and justification for not performing the same principal on IPC samples (DS, dilution buffer or bulk DP).

Query prior to European Medicines Agency – Questions and answers for biological medicinal products (September 2023) – Low Endotoxin Recovery, Endotoxin Masking Effect

Query # 2 – EU Query – 2023

For endotoxin release testing of the DP, detailed data on **sample hold-time studies** are requested to ensure absence of Low Endotoxin Recovery (LER) effect.

European Medicines Agency – Questions and answers for biological medicinal products (September 2023)

Low Endotoxin Recovery, Endotoxin Masking Effect

- *First publication did not define LER as manufacturing or sample hold-time*
- *Current – "*LER studies should reflect conditions relevant for the manufacturing process, including relevant temperatures, and should reflect potential hold times during manufacture."

PDA TR82 defines LER

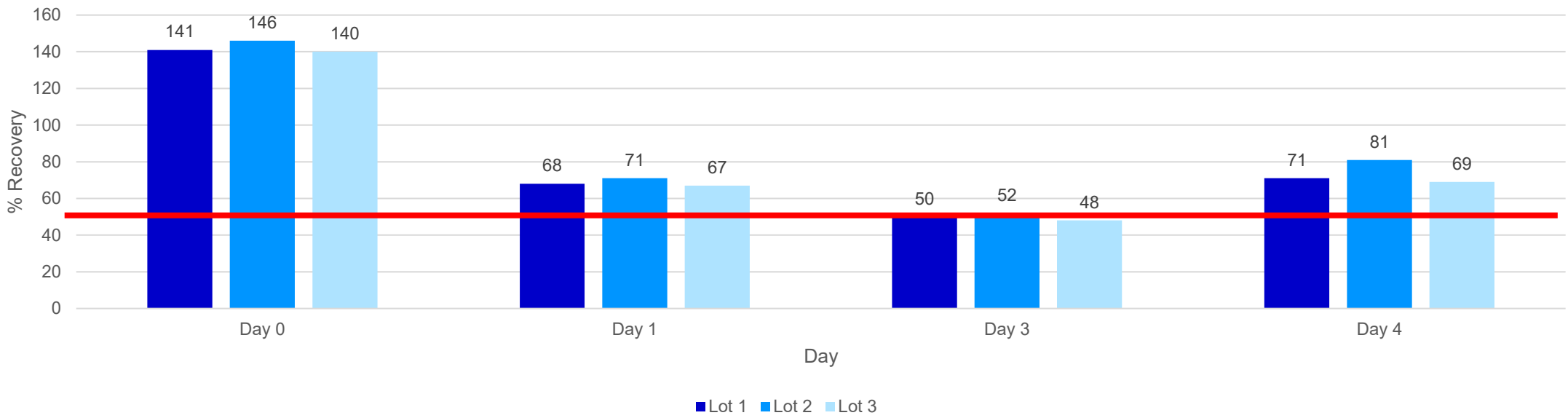
"LER hold-time studies should determine whether a product has LER under conditions which are relevant for the **manufacturing process"**

Query # 3 U.S. FDA – 2023

Results of the LER …. **drug product (DP) may mask endotoxin**, as evidenced by **the 50-52% recovery** of the endotoxin spike for DP batches 1 and 2 and **failure of DP batch 3 to meet acceptance criteria by Day-3 post-endotoxin spiking**….

Repeat the LER Study with testing time points after preparation and **every 24 hours thereafter**. Please refer to **PDA Technical Report No. 82 for guidance.**

Query # 3 – U.S. FDA – 2023



PDA TR82 – "A minimum of four time-points must be conducted to ensure valid and accurate results. **Two consecutive data points falling** below 50% recovery indicates LER. If the **last time-point** results in a **recovery of less than 50%, additional time-points should be tested** to aid in the analysis of LER endotoxin masking and to ensure that the LER result is accurate."

Query # 3 U.S. FDA Follow-up – 2023 cont.

Post Market Commitment

Conduct a LER study. The study should be conducted **from the beginning of drug substance thaw to the maximum time allowed for release testing**. In case the study shows endotoxin recoveries **below 50%** at process relevant conditions, develop an alternative endotoxin method to mitigate LER.

PDA TR 82

"Validation of **maximum quality control (QC) sample storage time**, that is, the maximum allowable time between sampling and testing, **is not in the scope of this technical report and should be conducted separately**." "if a step is conducted at 2–8 °C another at room temperature, the LER hold-time study should **be performed at room temperature** …LER hold-time study should be conducted for the combined longest process- and hold-times. Process step 3 **(2-8°C) is not considered** because LER would be detected during the room temperature hold-time study"

Query # 4 – EMA Query – 2024

? The **drug substance and drug product formulations** contain a surfactant and a chelator. Thus, low endotoxin recovery (LER) should be investigated.

European Medicines Agency – Questions and answers for biological medicinal products (September 2023)

"For **product formulations** that contain a combination of a surfactant (e.g. polysorbate) and a chelator (e.g. EDTA, citrate, phosphate, histidine), studies investigating Low Endotoxin Recovery (LER) should be submitted in the MAA dossier."

Query # 5 U.S. FDA (2025)

Provide the endotoxin recovery study report. Indicate whether the **endotoxin detection method used** was the gel clot method used on the routine test and clarify the **type of endotoxin used for the study** (**vendor product number of the control standard endotoxin (CSE) used**). This information is necessary because **some CSE marketed for endotoxin recovery studies are not susceptible to endotoxin masking**.

Queries Misaligned with Guidance

LER Studies for In-Process and Drug Substance

Inclusion of lab sample hold time with LER Study

One middle time point below 50% recovery fails LER

Timepoints at preparation and every 24 hours after

Not all CSE can be used for LER studies

Impact of Misalignment

LER studies not aligned across products → Confusion on how to conduct studies in the future

Slows BLA filings → May impact patients' access to medicines

- Pfizer was involved with several industry forums (PDA, BPOG, etc.) and worked directly with FDA on best practices for LER study design
- Pfizer is currently involved with update of PDA TR82

- Continue to file LER only with FDA, CDER and EMA
  - Supply data to other countries only if asked
- PDA TR82 is the only Guidance for LER studies – continue to follow guidance for studies and defend adherence to TR82

Thank You