Analytical Strategy & Operations

Overcoming Low Endotoxin Recovery from Theory to Practice – Case Studies

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Agenda

- Low Endotoxin Recovery Overview:
  - What is Low Endotoxin Recovery (LER)?
  - Current guidance
  - How are studies performed and when should they begin?
- LER Case Studies – Simplistic v. Complex

What is Low Endotoxin Recovery (LER)?

- LER was first introduced at the 2013 PDA Annual Meeting in Orlando, FL by Chen and Vinther (Genentech/Roche)

- LER is a failure to detect added/spiked endotoxin in undiluted product over time using the compendial Bacterial Endotoxin Test (BET)

- As a result, in 2013 US FDA's Center for Drug Evaluation and Research (CDER) began requesting LER studies for all new biologic products (i.e., all new CDER-regulated BLAs)

PDA Technical Report 82 LER

- PDA TR 82 was published in 2019 to provide guidance for industry when performing LER studies

- LER is defined as the inability to recover ≥50% activity over time when a known amount of endotoxin is added to undiluted product. LER cannot be overcome by dilution
  - In addition, the TR states that “LER occurs when two consecutive time points fail to achieve ≥50% recovery”

- LER studies should focus primarily on protein drug products (DP)

LER Study Overview per PDA TR 82

1. A known concentration of either Reference Standard Endotoxin (RSE) or Control Standard Endotoxin (CSE) is spiked into undiluted drug product and LAL Reagent Water (LRW)

2. Endotoxin spiked product/LRW are incubated at “process relevant” time and temperature
   - PDA TR 82 defines process relevant steps as those that are most likely to have an impact on LER (e.g., additional of polysorbate plus chelator, hold times, open/closed process steps, etc.)

3. Samples are then assayed using BET to determine the amount of endotoxin recovered

4. Endotoxin recovery is then calculated by comparing the amount of endotoxin in the sample against the amount of endotoxin in the LRW control at Time 0

LER and Method Development

- Identifying an endotoxin method to overcome LER may be labor and time intensive

- Given these considerations, endotoxin method development begins as soon as possible (i.e., when drug product formulation and protein concentration are “locked”)

- LER study is provided at the time of BLA filing and endotoxin method for commercial testing site is verified using a method that overcomes LER

Case Study I:LER can be a non-issue, depending on the test method

- A new matrix was being evaluated for LER
  - Formulation and administration was considering during LER method development
  - LER process relevant conditions were evaluated in alignment with TR 82
    - PDA TR 82 defines process relevant steps as those that are most likely to have an impact on LER (e.g., addition of polysorbate, chelator, hold times, open/closed process steps, etc.)

- Kinetic chromogenic methods were initially developed
  - Testing was performed at a 1:100 dilution and a 1:50 dilution

Case Study I: LER Method Development Evaluation by Micro COE

- LER method development initially using kinetic chromogenic method

- Reverse spiking approach was used for all LER studies described (spike at 72 hours, then 48 hours, etc. until Time 0)
  - For reverse spike approach, all time points are tested/assayed at the same time.

Case Study I: LER Study Results for Untreated Samples

- Initial results with the kinetic chromogenic method and no sample treatment

Time	% Recovery	
	Matrix 1	Matrix 2
Time 0	89	92
T24	45	65
T48	41	59
T72	39	57

LER observed for Matrix 1 at all timepoints!

Case Study I: Sample Treatments and the Kinetic Chromogenic Assay

- Various commercially available sample treatments (Pyrosperse, Dispersing Agent, Cation Buffer) were used
  - Various concentrations of the sample treatment components were tried in each assay
  - All reagents were unsuccessful (recoveries remained ≤ 50%) using the kinetic chromogenic method

Case Study I: Can changing the test method overcome LER?

- Method development began using the kinetic turbidimetric method
  - Matrix 1 was tested at a 1:100 dilution
  - Matrix 2 was tested at a 1:50 dilution

Time	% Recovery			
	Matrix 1		Matrix 2	
	Rep 1	Rep 2	Rep 1	Rep 2
Time 0	125	128	129	178
T24	72	93	68	82
T48	73	94	64	70
T72	84	107	85	88

Success without sample treatment!

Case Study II: Overcoming LER

- Background
  - LER study was performed by Contract Test Lab (CTL)
  - Study was performed at **2-8°C** for 14 days
  - **No LER was observed**
  - Kinetic chromogenic method was used
  - Re-evaluation of process relevant conditions occurred

Case Study II: LER Evaluation by Micro Center of Excellence

- Formulation of product was evaluated per PDA TR 82

- The manufacturing process for the product was also evaluated to determine the “process relevant conditions” for the LER study
  - 180 hours at RT was used for LER studies going forward

Case Study II: LER Method Development - Step 1

- If we use the kinetic chromogenic method to assess LER under new evaluation conditions, do we observe LER?

- Samples were held for 3 days at RT and tested (reverse spiking approach) to get an initial “read” on the kinetic chromogenic method

- LER (recovery below 50%) was observed at RT by the 24-hour time point

Case Study II: LER Method Development - Step 2

- Can commercially available sample treatments be used to overcome LER?
  - Pyrosperse – unsuccessful
  - Dispersing agent – inconsistent results
  - Cation buffer (0.5 M $MgSO_4$, 1 M Tris buffer) – mixed results depending on concentration used

Case Study II: LER Method Development - Step 3

- What other reagents have been used to overcome LER? Can we combine reagents to improve endotoxin recovery?
  - Additional reagents (various albumins, $CaCl_2$, dodecanoic acid, etc.) were also tested to determine if they help overcome LER
  - Approach resulted in Micro COE analyzing > 80 sample treatments using two endotoxin techniques

Case Study II: Overcoming LER

- The sample treatment that was successful to overcome LER utilized 5% Cation Buffer, 4% Pyrosperse, and 0.5 mM Sodium Octyl Sulfate using the kinetic turbidimetric method

- As per PDA TR 82, LER was not observed

Time	% Recovery		
	Rep 1	Rep 2	Rep 3
Time 0	87	91	79
48 hour	51	57	56
Day 7	52	52	49
Day 8	50	53	54
Day 9	69	68	60

Case Study II: Enabling the New Method at the CTL

- BMS Micro COE first verified the new method that overcomes LER
- Method was provided to CTL and our group supported method verification during site visit
- Method is now in place at CTL

In Conclusion

- LER can be overcome; however, the path forward can be unpredictable and complex
- LER must be assessed per product
- In theory, LER studies may seem simplistic; however, in practice, identifying a sample treatment to overcome LER can be a scientific challenge


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Thank you

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Back-Up

Case Study III: LER can be a non-issue, depending on the matrix

- A new matrix was being evaluated for LER
  - Formulation was considered during LER method development
  - LER process relevant conditions were evaluated in alignment with TR 82
  - Process relevant conditions = room temperature (RT) for 320 hours

- Kinetic turbidimetric methods were initially developed
  - Testing was performed at a 1:20 dilution

Case Study III: LER Method Development Evaluation by Micro COE

- Reverse spiking approach was used for all LER studies described
  - For example, Day 14 samples are spiked first and Day 0 samples are spiked last. All time points are tested/assayed at the same time.

- Method development proceeded with kinetic turbidimetric assay

Case Study III: LER Study Results for Untreated Sample Matrix

- Initial results with the kinetic turbidimetric method and no sample treatment, indicated that all recoveries were > 50%

Time	% Recovery		
	Rep 1	Rep 2	Rep 3
Time 0	74	75	82
Day 5	62	67	70
Day 11	68	71	73
Day 14	85	80	92

No LER is observed!

Case Study III: Method Verification and Transfer to Contract Test Lab (CTL)

- Method verification was first performed in-house by Microbiology Center of Excellence
  - All Product Positive Control (PPC) values ranged between 91-141%

- Method was verified at the CTL, enabling performance of the assay at CTL