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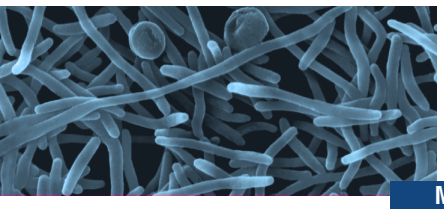

MICROBIAL SOLUTIONS

Low Endotoxin Recovery Hold-Time

Key Points:

- Low endotoxin recovery, or LER, describes the inability or failure to detect spiked endotoxin (purified LPS) levels in drug products.
- LER hold-time studies are used to help determine if a product has LER under conditions that correlate to potential hold-times that may occur within the manufacturing process.
- Two types of methods are used to perform LER testing: the chronological method or the reverse spike method.
- There is no data to support the claims that LER is, or ever has been, a patient safety risk.

Overview/Abstract

LER hold-time studies and QC sample storage hold-time studies are separate entities. LER hold-time studies are currently only requested for Center for Drug Evaluation and Research (CDER) Biologics Licensing Applications (BLA), whereas QC sample storage hold-time studies may be applicable to any product that is tested for endotoxin.[1] The US Food and Drug Administration (FDA) and European Medicines Agency (EMA) request that applicants submitting a BLA provide LER hold-time studies to determine any potential effect the sample matrix may have on endotoxin in drug products spiked with endotoxin using the Limulus amebocyte lysate (LAL) test.

Introduction to Low Endotoxin Recovery (LER) Hold-Time Studies

Low endotoxin recovery, or LER, describes the inability or failure to detect spiked endotoxin (purified LPS) levels in drug products when tested using an international compendial bacterial endotoxins assay using the LAL reagent. LER hold-time studies are not part of compendial endotoxin method suitability testing but are defined as supplemental studies. True LER hold-time studies measure the loss, if any, of endotoxin activity from time point zero (T0) up until the specified duration in the procedure.

LER-related loss of endotoxin activity has mainly been seen in drug products containing a protein formulated in a chelating agent with polysorbate. This LER observation led to speculation by some that if the recovery of purified endotoxin (also known as purified lipopolysaccharide, or LPS) is suppressed, then the activity of contaminating and native endotoxins may also be suppressed. This speculation also led some to the conclusion that the loss of endotoxin activity in finished drug product could result in a false negative LAL drug product released into market with the idea of major patient safety concern.[2] What came as a result was the US FDA's requested LER study procedure for a specific product's BLA to be tested for the LER effect, to ensure that the endotoxin assay method can overcome LER interference.

LER hold-time studies are used to help determine if a final product has LER under conditions that correlate to potential hold-times that may occur during drug product manufacturing. While a QC sample storage hold-time study is very similar to a LER, it is not part of a BLA submission. It is designed to guide QC labs in their standard operating procedures (SOP) to determine the amount of time a sample can be held for before testing. These strict time limits and conditions of storage are what stem from the endotoxin hold-time studies.

Methods of Hold-Time Studies

The basis of a hold-time study is to measure the endotoxin activity over time in spiked samples to assess whether the

product loses or gains endotoxin activity, and to establish a time frame and conditions of storage in which the specific product must be tested for endotoxin. Hold-time studies are not only important for establishing these limits, but regulators are also beginning to look at these studies as another source of assuring patient safety.

LER hold-time studies for a CDER BLA should be executed to a company's specific procedure or protocol, which has been approved by Quality Assurance. These protocols and procedures should include all acceptance criteria.

The two types of methods that are used to perform the testing are the chronological method or the reverse spike method.

a. Chronological Approach: This method is performed by spiking the sample and testing that sample at specific time points until the duration has ended, for example, Time Point Zero, 1 Day, 3 Days, 7 Days, and 14 Days. In order to avoid potential manipulation of the same sample container with each day's testing, separate aliquots may all be prepared at Time Point Zero and tested at their appropriate interval.

b. Reverse Method: Samples are prepared from the same original container and multiple, individual containers are spiked with endotoxin in reversed time. An example is that 5 aliquots are pulled from the original container, then one aliquot is spiked first as Day 14 sample, the second aliquot is spiked 7 days later as Day 7 sample, the third aliquot is spiked 4 days later as Day 3 sample, the fourth aliquot is spiked 2 days later as Day 1 sample, and the fifth aliquot is spiked 1 day later as Time Point Zero sample. All the time point samples are then tested at the same time. There is minimal day-to-day variability associated with the standard curve with the Reverse Method.

There are pros and cons to both methods, so the potential risk should be evaluated prior to choosing which method to use.

Considerations and Recommendations for Hold-Time Studies

- The samples in hold-time studies should be in their original containers, as they would be stored in real-world conditions.
- The compendial BET method used should be the same method that will be used for routine testing for those samples, as well as the same vendor for the LAL.
- The samples should be stored at product filling temperature.
- All testing reagents must be qualified according to the harmonized BET chapters prior to use, and accessories must be free of detectable endotoxin and non-interfering to the assay.
- Test at least three different lots of product to assure acceptance even with lot to lot variability, although less/more lots may be tested depending on the circumstances.
- The volume of the spike should not exceed 10% of the total volume.
- The final endotoxin concentration in the undiluted drug product is a muiltiple that uses the spike level at the mid-point of the standard curve and the validated dilution of the drug product.
  Example:
  5, 0.5, and 0.05 Standard Curve
  Validated product dilution = 10
  10 x 0.5 EU/mL = 5 EU/mL
- Use reference standard endotoxin (RSE) or control standard endotoxin (CSE) that has been calibrated against the RSE. This use of a purified LPS standard is considered worst-case scenario and FDA recommended.

Considerations and Recommendations for Hold-Time Studies, Continued

- Decide the appropriate time points at which the product will be tested, the duration of the study, and the temperature at which spiked product will be held at. The amount of time for the study should be the same time the product takes to go from manufacturing to QC. A minimum of 4 time points is recommended to be conducted to ensure valid and accurate results. A recommended storage temperature of 20-25 °C may also be appropriate as manufacturing processes are rarely performed at refrigerated/frozen temperatures, and the LER effect is thought to proceed more rapidly at room temperature than refrigerated/frozen temperatures.[3]

- In addition to spiking the product, LAL reagent water (LRW) should also be spiked to an equal amount of endotoxin as the product and diluted/prepared in the same manner as the product. This LRW control can be used as the comparative marker when determining the reduction in endotoxin.

Interpretation

Samples that do exhibit LER effect in hold-time studies typically decline to less than (<) 50% over the time points in relation to the comparative marker, and two consecutive time points that result in a recovery of <50% indicates LER.

Hold-time studies continue to be an important part of an organization's manufacturing and release testing programs. When executing a hold-time study, consider the method you would like to use, reagents, equipment, storage conditions, and the duration. With proper planning and execution, hold-time studies can be completed without any major setbacks, and what has been described as LER is linked to biological products in a specific matrix. The FDA and regulatory agencies have LER-related concerns, but there is no data to support the claims that it is, or ever has been, a patient safety risk.

References

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