Hold-Time Studies and Batch Testing: Considerations to Maximize Endotoxin Efficiency

Application Note

Introduction

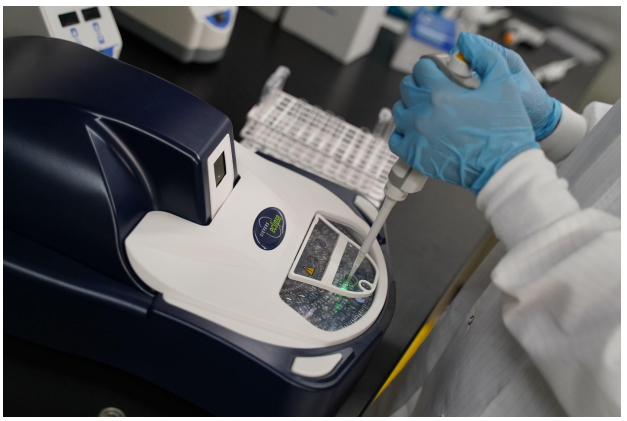
This application note describes “batch testing” and sample hold-times as they refer to endotoxin testing and explains how simple studies can be performed to maximize efficiency. Not all quality control or research and development labs are high throughput labs, meaning they only receive a few samples per day or a few samples per week that require Limulus Amoebocyte Lysate (LAL) testing. Therefore, in order to make LAL testing more economical and more efficient, some labs “batch” or hold their samples until they have enough on hand to utilize a full 96-well plate or a Sievers* Eclipse microplate. This allows them to consolidate their testing and not waste LAL, an expensive reagent.

The Importance of Determining Appropriate Hold Times

While batch testing can improve efficiency and reduce costs, labs often have internal procedures outlined in their Standard Operating Procedures (SOPs) that require samples to be tested for endotoxin within a specified time frame (example would be 24 hours upon receiving or sampling). These timeframes can limit the ability of small labs to batch or hold their samples, and may be more restrictive than they need to be. Testing samples within a certain time frame is not a regulatory requirement, but it is a general Good Manufacturing Practice (GMP) requirement to establish an appropriate time frame to test samples that results in no loss of endotoxin if there were to be any present.These are referred to as hold times. Thus, performing hold time studies and determining appropriate hold times will provide information critical to balancing needs related to quality, costs, and efficiency. Hold time studies are also useful when sending samples to a contract lab for analysis in order to determine the time required for results to be obtained.

Conducting Hold Time Studies for Endotoxin Testing

To get the most out of the LAL reagent, cost of consumables, and utilization of a microplate, one can perform a simple hold time study to determine and confirm that there would be no loss of endotoxin if a sample is stored for batch testing longer than 24 hours. Hold time studies are designed to guide QC labs in their SOPs to determine the amount of time a sample can be held for before testing.[1] An important attribute to a hold time study is a sample storage container. For endotoxin testing, samples should be collected and stored in an appropriate container that does not interfere with the LAL test or adsorb endotoxin. It is well known that polypropylene containers adsorb endotoxin, and the best sample container for this purpose is polystyrene or borosilicate glass.



A hold time study should be conducted with a minimum of four time points to ensure valid and accurate results.[2] These can be Day 0, Day 1, Day 3, and Day 7. Water samples, in-process samples, raw materials, and even final drug product samples can be utilized for a hold time study to determine the appropriate time that one can store samples prior to testing. Each sample that is intended to be tested for the hold time study will be spiked with a known concentration of endotoxin. It is recommended that a user spikes with a minimum concentration on the midpoint of the standard curve, however, the higher the concentration, the more likely the user is to recover within a two-fold of that concentration. As an example, the Parenteral Drug Association (PDA) Technical Report No. 82, TR 82, states to spike with 5 EU/mL.[2] These samples will be tested at all of the timepoints suggested to measure and confirm there is no loss of endotoxin.

The main concern of holding samples without conducting such a study is a potential false negative in a sample over time that could lead to patient safety risks. Once confirmed that there is no endotoxin loss over 7 days, a lab can implement a new hold time or testing requirement in their SOP that allows them to hold samples up to 7 days in order to test all samples at once instead of a few samples throughout the week.

Efficiency Gains with the Sievers Eclipse

The Sievers Eclipse is a fully compliant endotoxin testing platform that aligns with the compendial requirements outlined in USP <85>, EP 2.6.14, and JP 4.01 and contains an embedded standard curve with five (5) concentrations made with Reference Standard Endotoxin (RSE) ranging from 50-0.005 EU/mL in triplicate, along with embedded positive product controls (PPCs) for every sample. It is a high throughput endotoxin solution where current and potential users could benefit from maximizing the amount of samples to be tested during one given testing session. Users who have or will implement an extended hold time period for samples can utilize the freeze-thaw method of LAL reagent when testing on the Eclipse. It has been demonstrated that freezing LAL after initial reconstitution and using it at a later time for sample testing on the Eclipse provides consistent results.[3]

Conclusion

As it is a GMP recommendation to establish proper hold times for sample testing, users can execute a simple study to maximize gains in the QC lab in terms of sample throughput with minimal hands-on time.

References

1. H.Skalski. Low Endotoxin Recovery Hold-Time Study Considerations. Charles River Laboratories, April 2020.
2. PDA Technical Report No. 82. Low Endotoxin Recovery. PDA, 2019.
3. LAL Reagent Storage Evaluation Using the Sievers Eclipse BET Platform. Sievers Analytical Instruments, 2022.

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