2024 PDA Pharmaceutical Microbiology Conference | Oct 07 - Oct 09, 2024 | Washington, DC

Good practice in LER hold time study: the choice of the endotoxin

by Alessandro Pauletto, Christian Faderl, Holger Grallert, Gregory Devulder, Luca Di Bello and Kevin L. Williams, bioMèrieux

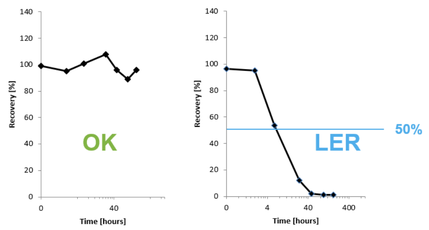
INTRODUCTION

For many years, there was ongoing debate over which type of endotoxin—naturally occurring endotoxin (NOE) or standard endotoxin (CSE/RSE)—was most suitable for use in LER (low endotoxin recovery) hold time studies. This debate ultimately led to the decision, as reflected in PDA TR82, to standardize the use of CSE and RSE, despite not fully considering the potential differences between various CSEs. In this study, we analyze the differing behaviors of several CSEs (and RSE) across a range of matrices relevant to the biopharmaceutical field.

CONCLUSION: THE USE OF CSE AND RSE IN LER HOLD TIME STUDIES WAS INITIALLY DRIVEN BY THE NEED FOR STANDARDIZED EXPERIMENTAL RESULTS, BUT WHILE RSE IS A PRIMARY STANDARDS WITH SPECIFIC REGULATORY REQUIREMENTS, CSEs ARE SECONDARY STANDARDS LACKING UNIFIED REGULATIONS, WHICH MAY IMPACT THE CONSISTENCY AND RELIABILITY OF LER STUDY OUTCOMES.

BACKGROUND

Low Endotoxin Recovery (LER) refers to the phenomenon where endotoxins exhibit a reduced or undetectable response in the LAL (Limulus Amebocyte Lysate) assay after being exposed to certain conditions or matrices, such as proteins, buffers, or other pharmaceutical formulations. This reduced detection does not indicate the absence of endotoxins but rather a masking effect, where the endotoxins are not effectively recognized by the assay.



According to PDA Technical Report 82, hold time studies should be conducted by spiking the undiluted sample with Control Standard Endotoxins (CSE) or Reference Standard Endotoxins (RSE). The use of Naturally Occurring Endotoxins (NOE) is acceptable, but only as supplementary studies. This guideline emerged after extensive debate on the use of NOE, ultimately favoring endotoxins that are more standardized in the manufacturing process to ensure more reproducible studies. However, this approach did not consider the heterogeneity of different CSEs available on the market, which may come from different bacterial strains and potentially involve varying extraction, production, and formulation processes.

In this study, we analyzed potential differences in reactivity that may arise when conducting an LER hold time study using five different CSEs from various endotoxin detection reagent manufacturers, along with the USP RSE. The study was conducted across six different matrices relevant to biopharmaceutical production, utilizing two different reagents: rFC ENDOZYME® II (bioMèrieux SA) and Kinetic-QCL™ (Lonza). The hold time study was performed under two different incubation temperatures to assess temperature-related effects on endotoxin recovery. The goal of the study was to evaluate the overall impact of these various factors on the results of a hold time study, providing insights that could enhance the reproducibility and reliability of endotoxin detection in complex biopharmaceutical matrices.

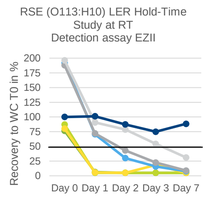
- 10 mM Citrate + 0.05% Polysorbate 80
- 10 mM Citrate + 0.025% Polysorbate 80
- 10 mM Citrate + 0.01% Polysorbate 80
- 1x PBS + 0.05% Polysorbate 80
- 1x PBS + 0.025% Polysorbate 80
- 1x PBS + 0.01% Polysorbate 80
- Water Control

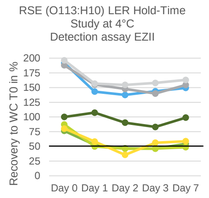
rFC ENDOZYME® II (bioMèrieux SA) = EZII
Kinetic-QCL™ (Lonza) = KCA

References:

- PDA TR 82 Low Endotoxin Recovery (2019)
- USP<1085> Guidelines on the Endotoxins Test
- FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers (2012)
- Masking of endotoxin in surfactant samples: Effects on Limulus-based detection systems - Johannes Reich, Pierre Lang , Holger Grallert , Hubert Motschmann - Biologicals 2016 Sep;44(5):417-225

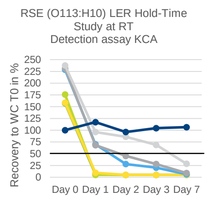
DATA

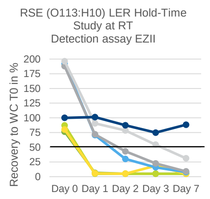




Matrix Effect: In this initial experiment, we assessed the impact of the six different matrices on a sample spiked with RSE at a concentration of 10 EU/ml. The results are presented as percentages relative to a control water sample processed under the same conditions. The hold time study was conducted at room temperature. Detection method used ENDOYME II (bioMèrieux SA)

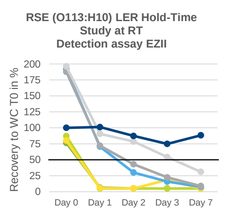
Temperature Effect: A hold time study is typically conducted at two different temperatures: room temperature and 2-8°C, depending on the manufacturing process in place. In this experiment, we replicated the previous conditions but varied the storage temperatures. Specifically, we conducted the study at 2-8°C to assess the potential impact of lower temperatures on the results. This comparison helps determine the effect of temperature on endotoxin recovery in different matrices.

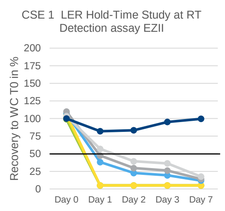


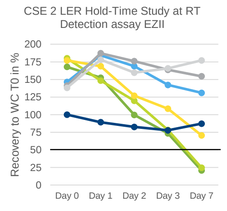


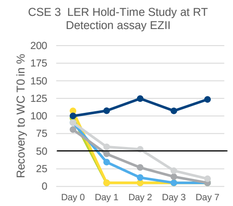
Detection method effect: PDA TR 82 highlights the potential impact that different detection methods may have on hold time results. For this study, we compared a rFC against results obtained by a Kinetic Chromogenic Assay (KCA).

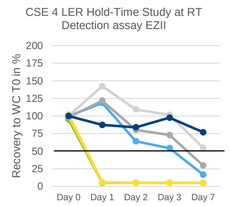
Endotoxin effect: In this study, we compared the results obtained using five different CSEs available on the market from main suppliers for endotoxin reagents against those obtained using the USP-RSE. The detection method was ENDOZYME® II (bioMèrieux), with room temperature storage on the same matrices and the same spiking level as in previous studies.

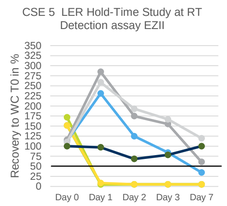












RESULTS

RESULT BELOW 50% AT 7 DAYS?			
buffer	RSE (O113:H10)	RSE (O113:H10)	RSE (O113:H10)
	RT EZII	4°C EZII	RT KCA
10 mM Citrate + 0.05% Polysorbate 80	YES	NO	YES
10 mM Citrate + 0.025% Polysorbate 80	YES	DOUBT	YES
10 mM Citrate + 0.01% Polysorbate 80	YES	NO	YES
1x PBS + 0.05% Polysorbate 80	YES	NO	YES
1x PBS + 0.025% Polysorbate 80	YES	NO	YES
1x PBS + 0.01% Polysorbate 80	YES	NO	YES

The PDA Technical Report highlights various factors that can influence the detection of LER phenomena. Many studies have demonstrated different reactivity within the same matrix at different storage temperatures. Our data align with these previous findings. Additionally, we confirmed that, for the samples under investigation, the rFC reagent exhibited reactivity substantially like that obtained with a kinetic chromogenic LAL reagent. The table below presents the results in a simplified form for easier comparison. LER is considered present when the value obtained on the 7th day is less than 50% of the reference value obtained from a similarly treated water sample.
The matrices used in this study are those previously shown to exhibit LER issues. Our findings confirm those seen in earlier studies.

RESULT BELOW 50% AT 7 DAYS?						
buffer	RSE (O113:H10)	CSE 1	CSE 2	CSE 3	CSE 4	CSE 5
	RT	RT	RT	RT	RT	RT
10 mM Citrate + 0.05% Polysorbate 80	YES	YES	YES	YES	YES	YES
10 mM Citrate + 0.025% Polysorbate 80	YES	YES	YES	YES	YES	YES
10 mM Citrate + 0.01% Polysorbate 80	YES	YES	NO	YES	YES	YES
1x PBS + 0.05% Polysorbate 80	YES	YES	NO	YES	YES	YES
1x PBS + 0.025% Polysorbate 80	YES	YES	NO	YES	YES	NO
1x PBS + 0.01% Polysorbate 80	YES	YES	NO	YES	NO	NO

This table presents the simplified results of the comparison performed on the same matrices during a hold time study at room temperature using the recombinant ENDOZYME® II reagent for detection. The differences in results obtained using different CSEs compared to the RSE are clearly evident. It is apparent that some CSEs exhibit differences in reactivity when compared to the RSE. Specifically, CSE 2 shows no LER issues for the PBS-based matrices and for the citrate matrix, although only at higher polysorbate concentrations. Interestingly, both CSE 1 and CSE 2 come from the same supplier. CSE 4 and CSE 5 also show different reactivity, though limited to certain PBS-based matrices. Citrate-based matrices exhibit similar reactivity, with the exception of CSE 2, which aligns with the results obtained using the RSE.

CONCLUSION

The use of CSE and RSE in LER hold time studies in the past was motivated by the need to obtain results based on standardized experimental conditions. However, this applies only to the concept of RSE as a primary standard. CSEs, on the other hand, are by definition secondary standard that are not subject to specific regulatory requirements, except as noted, for example, in USP 1085 or FDA Q&A. These are secondary standards that are verified against a primary standard to determine their potency. There are no regulations defining, for example, the strain, culture methods, or extraction and formulation processes. However, it is clear that these aspects can influence the structure of endotoxin and, consequently, its potential behavior in LER studies. The results of this preliminary study, which is still ongoing, confirm the hypothesis that different CSEs may affect the repeatability and reliability of LER study results due to the lack of a unified standard.