Bridging the Gap: A Comparison Study Between a Recombinant Cascade Reagent and Limulus Amebocyte Lysate

Melissa J. Cramer, Allan J. Romberg & Bradley C. Parish

charles river

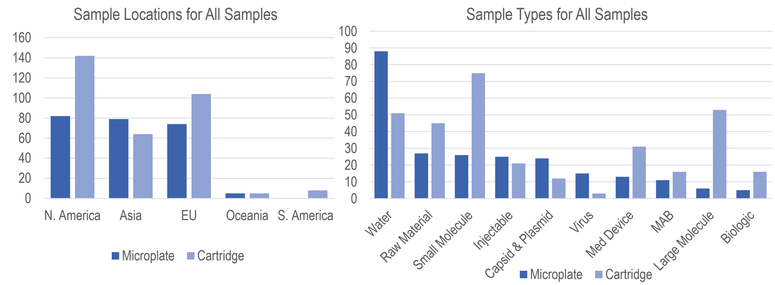
1 Introduction

Determining endotoxin levels in pharmaceutical products and medical devices is necessary to minimize the risk of a pyrogenic response and ensure patient safety. For four decades, the Limulus Amebocyte Lysate (LAL) test has been the gold standard for *in vitro* bacterial endotoxins testing (BET). With sustainability efforts and the advancements in technology, recombinant reagents have been developed as alternatives to animal-based tests. Before the LAL test can be replaced, it is key to determine that alternate methods and reagents demonstrate an equivalent performance to FDA-licensed compendial LAL reagents.

This study evaluated the performance of two recombinant cascade reagent (rCR) assays (microfluidic cartridges and microplate) to the respective LAL based assays with pharmaceutically relevant samples. A total of five hundred sixty-three (563) samples were utilized for comparative performance which included interference patterns and samples contaminated with natural environmental endotoxin (NEE). Statistical analysis of 134 endotoxin positive samples showed that the rCR assays were equivalent to the licensed LAL assays proving that the new rCR assays were able to detect endotoxin equally to the FDA-licensed biological assays, ensuring the same patient safety as LAL tests. This study utilized a comprehensive real-world dataset, containing naturally contaminated endotoxin samples, which evaluated the performance of rCR methods to traditional licensed LAL reagents.

2 Samples

Study participants could contribute to the comparative study in two ways; 1. Submission of samples or 2. Submission of paired data. A total of two hundred forty (240) samples were obtained from North America, Europe, Asia, and Oceania for microplate testing and three hundred twenty-three (323) samples were obtained from North America, South America, Europe, Asia and Oceana for cartridge testing.



3 Methods

Microplate Based Endotoxin Analysis – All samples were tested per each participant's validated LAL methods. Samples received were simultaneously tested for endotoxin using either Kinetic Chromogenic (KCA) or Kinetic Turbidimetric (KTA) and Recombinant Cascade (rCR) against a 5 – 0.005 EU/mL RSE standard curve. KCA and KTA were rehydrated with 3.2mL or 5.2mL respectively of Beta-Glucan Blocker and rCR was rehydrated with 1.7mL LRW. Each sample was examined in duplicate along with a duplicate positive product control. Each well was measured at 405nm for chromogenic reagents and 340nm for turbidimetric reagents. All plates were incubated at 37°C until the lowest standard crossed 0.1 delta OD. All results were analysed via EndoScan-V version 6.2.0. A linear regression model was used to interpolate sample OD values.

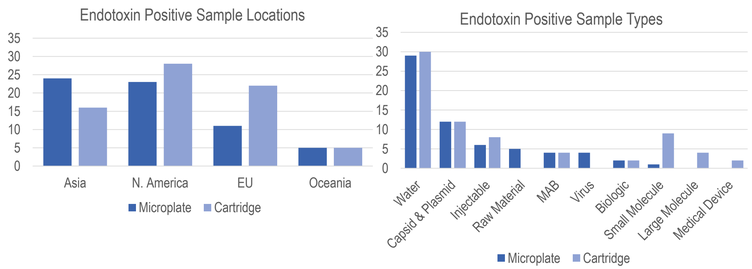
Cartridge Based Endotoxin Analysis – All samples were tested per each participant's validated microfluidic cartridge LAL methods. Each sample was diluted a minimum of 50% with Beta Glucan Blocker for LAL cartridges or LRW for rCR cartridges. Both LAL and rCR cartridges were at a sensitivity of 1.0-0.01 EU/mL. Twenty-five (25) microliter aliquots of each sample were loaded into the four sample reservoirs of the cartridge. The sample and spike values were calculated by interpolation of the internally archived standard curve using the reaction times. All data were analysed via EndoScan-V version 6.2.0.

4 Results & Statistical Analysis

Of the five-hundred sixty-three (563) samples included in the study, one-hundred thirty-four (134) samples demonstrated measurable endotoxin and were utilized in further analysis.

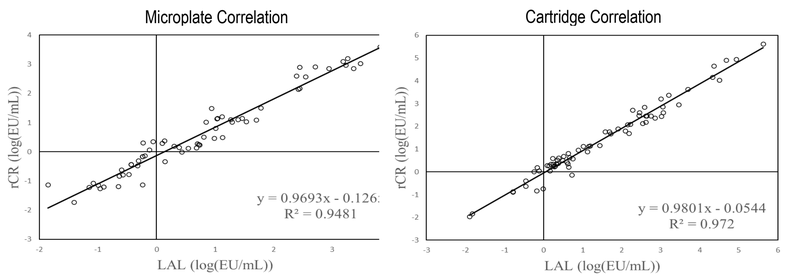
Test Method	Non-detectable Endotoxin	Detectable Endotoxin	Discordant Results
Microplate	162 (68%)	63 (26%)	15 (6%)
Cartridge	242 (75%)	71 (22%)	10 (3%)

The endotoxin positive samples were geographically diverse and comprised of a distribution of matrices for both microplate and cartridge assays.



The results from LAL and rCR testing were compared and shown as scatter plots. The rCR reagents demonstrate excellent agreement to the LAL reagents based upon the R^2 values and summary statistics. Given the accepted margin of error within LAL and endotoxin testing, the correlation indicates that the rCR microplate and cartridge assays are equivalent to the LAL counterparts.

Test Method	Microplate		Cartridges	
	Mean	Standard Deviation	Mean	Standard Deviation
LAL	0.770	1.385	1.432	1.614
rCR	0.620	1.379	1.349	1.605



The two one-sided test (TOST) method for testing equivalence of the rCR and LAL methods was performed using an ANOVA model for repeated measures after natural logarithmic transformation of the endotoxin values. A compound symmetry variance-covariance matrix was selected based on the Akaike Information Criterion (AIC), a mathematical method for evaluating how well a model fits the data it was generated from. The adequacy of this model was checked by analysing the residuals and the natural logarithmic transformation of the endotoxin values was applied to improve the normality of the residuals. The difference between the rCR and LAL methods and the two-sided 90% confidence interval were calculated. These were then back transformed using the anti-log to provide an estimate of the ratio of geometric means and the 90% confidence interval. This confidence interval was used to assess equivalence of rCR and LAL. If the confidence interval was contained entirely within the equivalence interval of (50%, 200%), equivalence was demonstrated.

All samples that demonstrated an endotoxin positive result for both rCR and natural LAL were included in the TOST statistical analysis to determine the overall performance and equivalence of the recombinant methods. The data demonstrates that the 90% confidence interval for rCR compared to LAL (69.7%, 84.6%) was contained entirely within the equivalence interval of (50%, 200%). It was concluded that rCR is equivalent to LAL.

Comparison	DF	Ratio of Geometric Means	90% Confidence Interval	Equivalent
rCR vs LAL	133	76.8%	(69.7%, 84.6%)	Yes

5 Summary

A comprehensive study comparing two formats of rCR methods (microplate and microfluidic cartridge) were compared to their LAL counterpart methods. The study included 563 pharmaceutically relevant samples, 134 of which were contaminated with natural environmental endotoxin. The samples demonstrated a high level of correlation between the two methods. The results demonstrated that rCR methods are generally suited for a wide variety of pharmaceutically relevant samples and demonstrate equivalent performance to FDA licensed compendial LAL reagents.