Validating rCR on a Microfluidic Endotoxin Testing Platform: A Multi-Matrix Approach

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Background

Recombinant Cascade Reagents (rCR) are emerging as a sustainable and innovative solution for Bacterial Endotoxins Testing (BET). By leveraging recombinant technology, rCR replicates the full Limulus Amebocyte Lysate (LAL) cascade without relying on horseshoe crab-derived resources, aligning with industry sustainability goals. As rCR adoption grows, it is crucial to ensure efficacy in detecting naturally occurring endotoxins from diverse Gram-negative bacterial sources.

This study evaluates PyroSmart NextGen® rCR performance by testing lipopolysaccharides (LPS) from various Gram-negative bacteria across three commercially available lots. Additionally, it compares two testing platforms: the Sievers Eclipse BET Platform and traditional 96-well microplates, assessing the consistency of endotoxin detection across serotypes and platforms while examining each method's performance characteristics.

Comparison Study

Study objectives:

- Demonstrate recovery of various endotoxin serotypes using ACC's PyroSmart NextGen® rCR on the Sievers Eclipse BET Platform and 96-well microplates with Molecular Devices SpectraMax® reader
- Challenge the Sievers Eclipse with rCR to demonstrate comparability to 96-well microplates

Test materials:

- LPS solutions created from microorganism strains obtained from Microbiologics KWIK-STIKs
- Reference Standard Endotoxin (RSE) lot R172R0
- PyroSmart NextGen® rCR provided by ACC

NextGen® rCR IFU

Samples

Crude lipopolysaccharide (LPS) solutions were created by isolating monocultures of the following microorganisms, obtained from LPS solutions prepared from lab-cultured Gram-negative microorganisms:

- *B. cepacia*, derived from ATCC® 25416™
- *E. coli*, derived from ATCC® 8739™
- *P. aeruginosa*, derived from ATCC® 10145™
- *R. pickettii*, derived from ATCC® 27511™
- *S. enterica*, derived from ATCC® 51741™
- *S. maltophilia*, derived from ATCC® 13636™

Cultures were suspended in Water for Cell Culture (WFCC), heated, vortexed, and filtered through 0.2µM syringe filters. Solutions were then diluted to achieve a target EU/mL response between 0.5-1.0 EU/mL. Tap water was also collected and diluted to the same target EU/mL. As a control for the LPS extraction method, WFCC was heated, vortexed, and filtered via the same methodology and tested for interference and contamination. The WFCC control showed the same characteristics as LAL Reagent Water (LRW) in assays. Reference Standard Endotoxin (RSE, lot R172R0) was prepared at concentrations of 50-0.005 EU/mL with serial dilutions and tested as a standard curve contemporaneously alongside all samples.

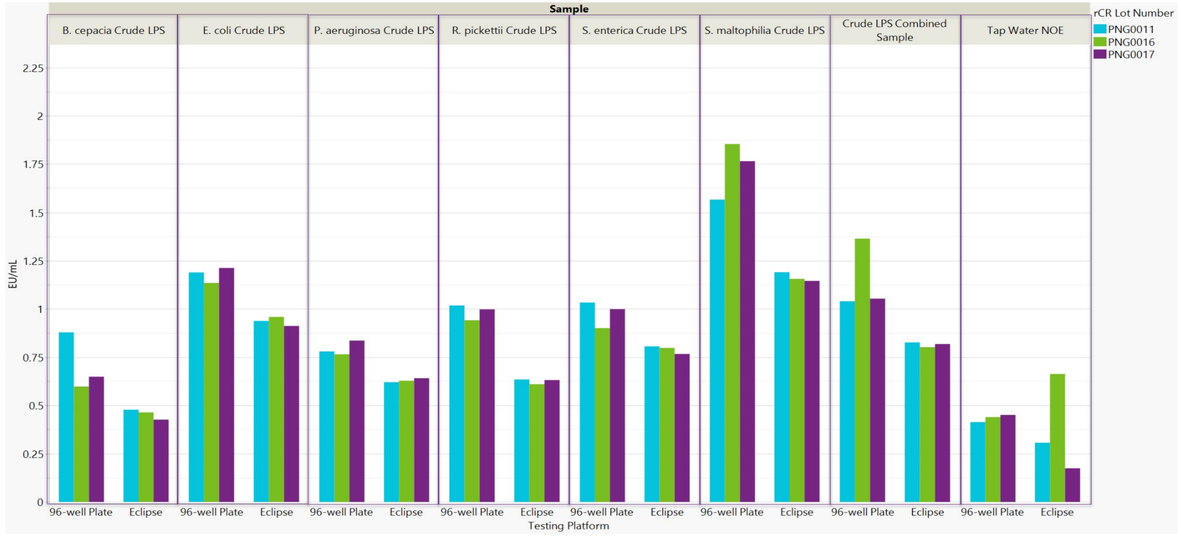
Conclusion

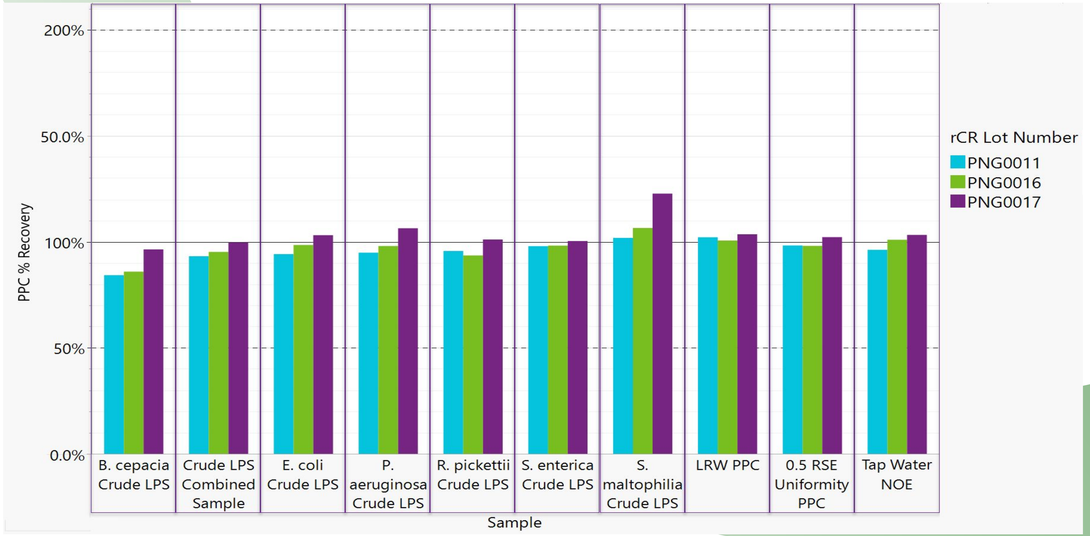
This study demonstrates that the Sievers Eclipse BET Platform delivers equivalent performance to traditional 96-well plates in detecting various bacterial endotoxin serotypes, including naturally occurring endotoxins. The findings confirm that the Eclipse effectively utilizes recombinant cascade reagents (rCR) to recover diverse endotoxins with high reliability.

The Eclipse's centripetal microfluidic technology streamlines endotoxin testing, offering significant time savings and reducing the potential for errors. At a sensitivity of 0.005 EU/mL, the average reaction time for RSE on the Eclipse was 1692 seconds, notably faster than the 2687 seconds observed on the SpectraMax.

This innovative platform simplifies BET assays, reduces variability, and ensures full compliance with compendial requirements, making it a robust and efficient solution for endotoxin testing. Key benefits include enhanced efficiency, reduced variability, and sustainable testing practices.

Results





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Resources:

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View a comparison of the Sievers Eclipse to other endotoxin testing platforms:

See rCR and LAL results for real-world pharmaceutical samples from Eli Lilly:

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