Sievers* Eclipse BET Platform Recombinant Reagent Analysis

APPLICATION NOTE

WATER TECHNOLOGIES

VEOLIA

Purpose

United States Pharmacopeia Chapter <86> permits the use of non-animal-derived reagents for endotoxin testing and includes methods that use both recombinant cascade (rCR) and recombinant Factor C (rFC) reagents. This Application Note demonstrates compatibility of the Sievers Eclipse Bacterial Endotoxins Testing (BET) Platform with rCR across various pharmaceutical products, providing a highly efficient, animal-free alternative to Limulus amoebocyte lysate (LAL) endotoxin testing.

Method suitability and optimization were performed for seven released commercial products using one lot of commercially available PyroSmart NextGen recombinant cascade reagent (rCR) versus the kinetic chromogenic LAL method with Chromo-LAL reagent on the Sievers Eclipse BET Platform. Both reagents are manufactured by Associates of Cape Cod. The seven products that were evaluated for this study are listed in **Table 1** below.

Table 1: Products Tested on Eclipse

Product	Manufacturer
Water For Injection	Cytiva HyPure
Lactated Ringers for Injection USP	B Braun
0.9% Sodium Chloride USP	Cytiva
5% Dextrose Injection USP	Baxter
Erythromycin Injection	Nexus Pharmaceuticals
Human Insulin Autoinjector (combination product) USP	N/A
Potassium Chloride Injection	Nexus Pharmaceuticals

Background Information

The Sievers Eclipse BET Platform uses the kinetic method for the analysis of a standard curve and up to 21 samples with positive product controls (PPCs). The Sievers Eclipse microplate contains pre-deposited Reference Standard Endotoxin (RSE) derived standards and PPCs, which allows the user to run up to a 5-point standard curve (50 – 0.005 EU/mL) with each assay, using only 1 mL of LAL.

Since commercially available rCR reagents behave like chromogenic reagents and utilize the LAL cascade mechanism, this study was aimed to determine if this rCR reagent would perform similarly to the traditional kinetic chromogenic LAL reagent with real life samples. Each product was tested on the Sievers Eclipse with both rCR and kinetic chromogenic reagent.

Sample Preparation & Experimental Test Plan

An initial screen of all products was performed by testing different dilutions in LAL Reagent Water (LRW) to determine which dilution achieved an optimal PPC recovery and overcame any sample interference. The dilutions analyzed included a 1:1, 1:10, 1:20, 1:50, and 1:100. See **Table 2** for products and dilutions analyzed with each reagent type after initial screening. Insulin, Erythromycin, and Potassium Chloride were tested at multiple dilutions during product testing to achieve more results and to overcome interference upon completion of product screening.

Acceptance Criteria for Standards and Products are as follows:

- Standard, Sample, and PPC CV%: ≤ 15%
- PPC Recovery: 50-200%
- R-Value: ≥ the absolute value of -0.980

Table 2: Product Dilutions

Product	Dilution
Potassium Chloride Injection	1:20, 1:50
Erythromycin Injection	1:20, 1:50
5% Dextrose Injection USP	1:10
Sodium Chloride USP	1:20
Lactated Ringers for Injection USP	1:20
Insulin Human Injection USP	1:10, 1:20, 1:50
Water for Injection	1:1

Results

Summarized results are listed below. **Table 3** and **Table 4** show the results for the standard curves and **Tables 5-10** show results for the samples.

Standard Curve Results

Table 3: 5-point Standard Curve Results with Chromo-LAL Lot: 337092 and Sievers Eclipse

Plate Number	R-Value	%CV Onset Time <15%	Negative Control >0.005 EU/mL
1	-0.999	Valid	Pass
2	-1.000	Valid	Pass
3	-1.000	Valid	Pass
4	-0.998	Valid	Pass

Table 4: 5-point Standard Curve Results with PyroSmart NextGen Lot: 2630003 and Sievers Eclipse

Plate Number	R-Value	%CV Onset Time <15%	Negative Control >0.005 EU/mL
1	-0.999	Valid	Pass
2	-0.999	Valid	Pass
3	-1.000	Valid	Pass
4	-0.999	Valid	Pass
5	-0.999	Valid	Pass

Sample Results

Potassium Chloride

Table 5: Potassium Chloride Results on the Sievers Eclipse (Replicates of three and five injections on each plate)

Plate #	Reagent/Dilution	PPC Recovery Average %	Sample CV% <15	PPC CV <15%	Results
1	Chromo-LAL (1:20) x3	107.9	Valid	Valid	<0.1 EU/mL
2	Chromo-LAL (1:20) x3	78.7	Valid	Valid	<0.1 EU/mL
3	Chromo-LAL (1:20) x3	91.7	Valid	Valid	<0.1 EU/mL
4	PyroSmart NextGen rCR (1:20) x3	84.9	Valid	Valid	<0.1 EU/mL
5	PyroSmart NextGen rCR (1:20) x3	68.6	Valid	Valid	<0.1 EU/mL
6	PyroSmart NextGen rCR (1:20) x3	74.1	Valid	Valid	<0.1 EU/mL
7	PyroSmart NextGen rCR (1:50) x3	102.5	Valid	Valid	<0.25 EU/mL
8	PyroSmart NextGen rCR (1:50) x3	101.5	Valid	Valid	<0.25 EU/mL
9	PyroSmart NextGen rCR (1:50) x5	97.1	Valid	Valid	<0.25 EU/mL

Erythromycin

Table 6: Erythromycin Results on the Sievers Eclipse (Replicates of three and five injections on each plate)

Plate #	Reagent/Dilution	Average PPC Recovery%	Sample CV% <15	PPC CV% <15	Results
1	Chromo-LAL (1:20) x 3	78.3	Valid	Valid	<0.002 EU/mg
2	Chromo-LAL (1:20) x3	83.3	Valid	Valid	0.003 EU/mg
3	Chromo-LAL (1:20)* x3	87.7*	Valid*	Valid*	<0.002 EU/mg*
4	Chromo-LAL (1:20) x3	71.3	Valid	Valid	<0.002 EU/mg*
5	PyroSmart NextGen rCR (1:20) x3	84.6	Valid	Valid	<0.002 EU/mg
6	PyroSmart NextGen rCR (1:20) x3	68.0	Valid	Valid	<0.002 EU/mg
7	PyroSmart NextGen rCR (1:20) x3	N/A**	Valid**	Valid**	N/A**
8	PyroSmart NextGen rCR (1:50) x3	120.4***	Valid/Invalid***	Valid/Invalid***	<0.005 EU/mg***
9	PyroSmart NextGen rCR (1:50) x5	98.3	Valid	Valid	<0.002 EU/mg

*One sample replicate contained Split Well

** Split wells occurred in all 3 replicates

***One replicate had a %CV > 15

5% Dextrose

Table 7: 5% Dextrose Results on the Sievers Eclipse (Replicates of three injections on each plate)

Plate #	Reagent/Dilution	Average PPC Recovery%	Sample CV% <15	PPC CV% <15	Results
1	Chromo-LAL (1:10)	123.4	Valid	Valid	<0.05 EU/mL
2	Chromo-LAL (1:10)	96.4	Valid	Valid	<0.05 EU/mL
3	Chromo-LAL (1:10)	102.9	Valid	Valid	<0.05 EU/mL
4	PyroSmart NextGen rCR (1:10)	80.2	Valid	Valid	<0.05 EU/mL
5	PyroSmart NextGen rCR (1:10)	75.2	Valid	Valid	<0.05 EU/mL
6	PyroSmart NextGen rCR (1:10)	95.6	Valid	Valid	<0.05 EU/mL

0.9% Sodium Chloride

Table 8: 0.9% Sodium Chloride Results on the Sievers Eclipse (Replicates of three injections on each plate)

Plate #	Reagent/Dilution	Average PPC Recovery%	Sample CV% <15	PPC CV% <15	Results
1	Chromo-LAL (1:20)	134.2	Valid	Valid	<0.1 EU/mL
2	Chromo-LAL (1:20)	101.5	Valid	Valid	<0.1 EU/mL
3	Chromo-LAL (1:20)	115.7	Valid	Valid	<0.1 EU/mL
4	PyroSmart NextGen rCR (1:20)	111.5	Valid	Valid	<0.1 EU/mL
5	PyroSmart NextGen rCR (1:20)	86.7	Valid	Valid	<0.1 EU/mL
6	PyroSmart NextGen rCR (1:20)	84.5	Valid	Valid	<0.1 EU/mL

Lactated Ringers

Table 9: Lactated Ringers Results on the Sievers Eclipse (Replicates of three injections on each plate)

Plate #	Reagent/Dilution	Average PPC Recovery%	Sample CV% <15	PPC CV% <15	Results
1	Chromo-LAL (1:20)	143.9	Valid	Valid	<0.1 EU/mL
2	Chromo-LAL (1:20)	122.8	Valid	Valid	<0.1 EU/mL
3	Chromo-LAL (1:20)	139.2	Valid	Valid	<0.1 EU/mL
4	PyroSmart NextGen rCR (1:20)	104.3	Valid	Valid	<0.1 EU/mL
5	PyroSmart NextGen rCR (1:20)	94.9	Valid	Valid	<0.1 EU/mL
6	PyroSmart NextGen rCR (1:20)	93.5	Valid	Valid	<0.1 EU/mL

Insulin Human Injection

Table 10: Insulin Human Injection Results on the Sievers Eclipse (Replicates of three, five, or six injections on consecutive plates)

Plate #	Reagent/Dilution	Average PPC Recovery%	Sample CV% <15	PPC CV% <15	Results
1	Chromo-LAL (1:10) x5	104.3	0.4 - 0.7	Valid	<0.05 EU/mL
2	Chromo-LAL (1:10) x5	87.0*	Valid*	Valid*	<0.05 EU/mL*
3	PyroSmart NextGen rCR (1:10) x3	52.4	Valid	Valid	<0.05 EU/mL
4	PyroSmart NextGen rCR (1:20) x3	55.6*	Valid*	Valid*	<0.1 EU/mL*
5	PyroSmart NextGen rCR (1:50) x3	101.2	Valid	Valid	<0.25 EU/mL
6	PyroSmart NextGen rCR (1:50) x5	79.3	Valid	Valid	<0.25 EU/mL

*One replicate contained Split Well

Water for Injection

Table 11: Water for Injection Results on the Sievers Eclipse (Replicates of three injections on each plate)

Plate #	Reagent/Dilution	Average PPC Recovery%	Sample CV % <15	PPC CV% <15	Results
1	Chromo-LAL (1:1)	120.4	Valid	Valid	<0.005 EU/mL
2	PyroSmart NextGen rCR (1:1)	110.2	Valid	Valid	<0.005 EU/mL
3	PyroSmart NextGen rCR (1:1)	114.1	Valid	Valid	<0.005 EU/mL

Conclusion & Recommendations

The Sievers Eclipse showed excellent compatibility with commercially available rCR PyroSmart NextGen across multiple product types with most real life products tested in this study. The Erythromycin and Insulin samples had several split wells with both reagents, most likely due to some sample interference. The rCR reagent can be optimized on Sievers Eclipse microplates should labs consider the use of non-animal derived reagents for endotoxin testing in alignment with USP <86>.

For future analysis, sample screening must be performed to determine optimal dilutions for each product when utilzing rCR reagents against LAL. More reagent lots, when commercially available, should also be tested to confirm accurate results and repeatability.