Comparability of Two LAL Systems

Pyros Kinetix® Flex tube reader/Pyrotell® -T vs Agilent Epoch 2 plate reader/Pyrochrome®

This technical white paper summarizes a comparability study conducted to assess whether the Epoch 2 plate reader used with ACC Pyrochrome® LAL chromogenic reagent yields performance comparable to, or better than, the Pyros Kinetix® Flex tube reader used with Pyrotell®-T for routine endotoxin testing under validated test parameters. The study was designed to evaluate system suitability, standard curve performance, replicate precision, and variability across multiple days, reagent lots, and analysts. The findings can be used to support customer decisions related to reader transition, method bridging, and onsite implementation.

Background

Instrument updates and replacements are a common part of the lifecycle of analytical methods used in pharmaceutical quality control. In addition, with the growing need for a modern, high-throughput systems for endotoxin testing, the plate reader platforms are better suited for end-to-end automation than tube readers. Agilent/BioTek Epoch 2 absorbance platereader is one of the platforms that can be used for as a replacement for tube readers – it is validated for use with multiple reagents (both LAL and recombinant cascade reagent) and is compatible with ACC's proprietary Pyros® eXpress software.

Both systems (tube and plate readers) are validated as part of their IOPQ and the reagents are both compendial reagents for endotoxin testing, it is expected that final sample results (adjusted for dilution) will be within 50 – 200% of each other (per USP <85>). This was documented in a publication[1] where over 100 unique water samples were tested on tube and plate reader. The primary objective of the publication was to determine whether equivalency can be observed between LAL and PyroSmart NextGen® recombinant cascade reagent on a statistically significant number of samples.

This study focuses solely on endotoxin standard performance compared directly for both systems to help determine what to expect from Epoch 2 as a replacement system and how to support the transition to the new system without introducing variability in performance.

Study Objective

The objective of this study was to compare performance between the PKFlex tube reader with Pyrotell-T reagent and the Epoch 2 plate reader with Pyrochrome reagent under routine-use conditions representative of large customer testing environments. The comparison was intended to support transition planning from PKFlex to Epoch 2 by assessing whether Epoch 2 could deliver equivalent or improved assay performance across critical analytical parameters.

Study Design

The study included two reader/reagent configurations tested in parallel using the same USP Reference Standard Endotoxin (RSE) preparations. Testing was performed over three days with two reagent lots each and two analysts. For each instrument, two standard curves were generated per run, resulting in total of 6 curves per system. This design enabled evaluation of within-run performance, across-day consistency, lot-to-lot effects, and analyst-to-analyst variability.

- Configurations compared: PKFlex/Pyrotell-T versus Epoch 2/Pyrochrome
- 6 standard curves per system
- 7 replicates per each concentration
- Two reagent lots
- Three study days
- Two analysts
- Shared RSE preparations and common supplies where applicable

Table 1. Test plan

	PKFlex/Pyrotell-T			Epoch 2/Pyrochrome		
Analyst	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	Batch 1	Batch 2		Batch 1	Batch 2	
B			Batch 1			Batch 1

Materials and Methods

Table 2. Materials and Equipment

Item	PN/catalog number	Lot number/CIN
PK Flex	PKF96-X	PKF96-9F-2678
Epoch 2	EPOCHNS	24101482
Pyroplates	CA961-10	19025090
Reaction tubes	TK100-10	224-25 *195-25 – test on 28 May 26
15mL centrifuge tubes	PN00517	14922018
250ul tips	PPT25	30404435
1000ul tips	PPT10	17004541
Pipette	P200	001347
Pipette	P100	001344
Repeater	N/A	001971
Vortex	N/A	389422
RSE	N/A	R172RO
Pyrochrome batch 1	CG1500-25	CKG0062
Pyrochrome batch 2	C1500-5	CK0049
Pyrotell-T batch 1	T0051-5	525-09-045-T
PYROTELL-T batch 2	T0051-5	523-01-001-T
Glucashield	GB051-5	1207101
Glucashield	GB051-5	1207120
LRW	WP10001	AL30822104

Pyrotell-T is an LAL reagent for a kinetic turbidimetric assay.[2]
Pyrochrome is an LAL reagent for a kinetic chromogenic assay.[3]
Both reagents are FDA-licensed and are used per USP <85> Bacterial Endotoxins Testing[4]

Acceptance Criteria

Comparability was assessed using routine onset assay conditions appropriate to each configuration. Key performance indicators included negative control separation, standard curve linearity, replicate precision (CV%) based on onset time, and variability of slope and Y-intercept. Acceptance criteria were derived from reagent validity requirements and internal expectations for photometric reagent performance.

With the growing need for a modern, high-throughput systems for endotoxin testing, the plate reader platforms are better suited for end-to-end automation than tube readers.

- Negative controls must have onset times greater than the lowest standard concentration.
- Standard curves must have an absolute correlation coefficient of at least 0.980.
- Replicate CV% (onset time) should be less than 15% (7 replicates).
- Slope CV% target: less than 10%.
- Y-intercept CV% target: less than 3%.

Results

See Table 3 and Table 4.

Discussion

Key Findings

The study showed that Epoch 2/Pyrochrome met all acceptance criteria and delivered highly consistent performance across all runs. By comparison, PKFlex/Pyrotell-T also performed acceptably overall but showed a small number of exceptions primarily at the lowest concentration of 0.001 EU/mL and in one slope variability assessment. These findings indicate that Epoch 2 offers a robust technical bridge for customers transitioning from PKFlex.

System Suitability

Negative controls met expectations for all Epoch2 curves and for 5 out of 6 PKFlex curves. Standard curve linearity passed

Table 3. Summary of results

Reader	Day	SC#	Onset times in seconds (mean of 7 replicates)					CV% (onset time)			
			Neg.	0.001	0.01	0.1	1	0.001	0.01	0.1	1
PKFlex	1	1	>7873	5386	2674	1588	1093	3.97	5.87	0.76	0.71
		2	7470	4668	2654	1577	1063	16.42	3.27	0.91	0.36
	2	1	>9821	6056	3330	2002	1351	10.75	2.51	1.22	1.09
		2	>9203	4995	3329	2033	1381	15.78	0.91	1.27	0.73
	3	1	>3858	3585	2469	1588	1095	2.88	1.81	2.39	1.16
		2	3830	4235	2605	1624	1111	3.88	5.49	1.79	2.15
Epoch 2	1	1	>5133	4139	2820	1671	924	2.30	3.01	2.29	1.70
		2	4845	4137	2894	1724	950	4.73	1.24	1.34	1.50
	2	1	>6621	4476	3005	1826	1083	4.29	1.58	1.35	2.43
		2	6421	4667	3026	1880	1105	7.26	2.85	4.10	3.85
	3	1	>4217	3882	2716	1700	1012	2.82	13.70	2.77	5.37
			>4447	4085	2865	1740	1018	1.42	1.63	1.74	2.92

Table 4. Comparison of Reference Values (obtained from Certificate of Analysis) and Study Results

Reagent	Reader Type	Mean Onset time for 0.001 EU/mL	Means of Standard curve descriptors			CV%		Specifications for CV%	
			Slope	Y-int.	R	Slope	Y-int.	Slope	Y-int.
Pyrotell-T 523-01-001-T	CofA (PKFlex)	5340s	-0.182	2.997	-0.996	1.3	0.7	<10	<3
	525-09-045-T	4821s	-0.203	3.053	-0.997	6.9	1.2		
Pyrotell-T 523-01-001-T	CofA (PKFlex)	5388s	-0.181	3.020	-0.995	5.3	1.1		
	Pyrotell-T	5526s	-0.202	3.120	-0.998	21	0.9		
Pyrochrome CKG0062	CofA (ELx808)	3747s	-0.200	3.010	-0.996	2.7	0.3		
	Epoch 2	4231s	-0.207	3.020	-0.997	2.6	0.5		
Pyrochrome CK0049	CofA (ELx808)	4588s	-0.210	3.040	-1.000	1.5	0.2		
	CKG0062	4572s	-0.210	3.050	-0.999	1.4	0.0		

across all datasets (see Table 4). These results demonstrate that both systems generated analytically suitable curves under the study conditions.

Precision and Variability

Replicate precision was acceptable overall for both systems; however, PKFlex showed isolated failures at 0.001 EU/mL, where two of 6 curves exceeded the replicate CV% criterion. There were no failures on Epoch 2 (see Table 3).

For inter-assay variability, slope and Y-intercept were evaluated as the standard curve descriptors dictating the back-calculated values (see Table 4). Epoch 2 met slope and Y-intercept CV% expectations for both Pyrochrome lots. PKFlex met Y-intercept variability targets for both Pyrotell-T lots, but one lot exceeded the slope CV requirement, indicating greater variability in that configuration.

Comparison against Reference Values

Both systems were also evaluated against historical performance of the reagent lots as obtained during release testing (see Table 4). While both PKFlex and Epoch 2 systems mirror the performance as obtained during release testing, there is a high degree of alignment between the plate reader reagent lot specific performance (although release testing was performed on BioTek/Agilent ELx808 plate reader, the predecessor of Epoch 2): mean of onset times, slope, Y-intercept, and correlation coefficient were all closely aligned.

This shows that Epoch 2/Pyrochrome performance is robust and repeatable and thus this system can serve as a reliable replacement configuration for customer applications.

Executive Summary

A side-by-side comparability study was performed using PKFlex/Pyrotell-T and Epoch 2/Pyrochrome configurations under routine-use conditions. 6 standard curves were generated per system across three study days using two reagent lots and two analysts. Results demonstrated that both systems met acceptance criteria for linearity, while Epoch 2 showed more consistent low-end precision and lower degree of curve-parameter variability to the Pyros Kinetix Flex system:

- Negative controls passed for all Epoch 2 curves and for 5 of 6 PKFlex curves.
- Correlation coefficient criteria met across all datasets.
- CV% (onset time):
  - PKFlex: isolated failures in replicate precision at 0.001 EU/mL
  - Epoch 2: met for all datasets
- Slope and Y-intercept CV% criteria:
  - PKFlex: met for one Pyrotell-T lot but failed slope CV% criterion for the other Pyrotell-T lot
  - Epoch: met for all datasets

Epoch 2/Pyrochrome met all criteria for all datasets and also this study data mirrored very closely data obtained for these reagent lots as obtained during release testing.

Overall, the study supports Epoch 2/Pyrochrome as a suitable replacement configuration for routine use, subject to customer-specific onsite validation requirements. For customers planning a transition from PKFlex, these results demonstrate a strong technical basis for bridging and implementation.

Customer Implications

For customers considering a transition from PKFlex to Epoch 2, these results provide practical evidence that the new reader/reagent configuration can support routine endotoxin testing with strong analytical performance. The data suggests that customers may expect equivalent or improved consistency, particularly at the low end of the standard curve where variability is often most visible. As with any method transition, onsite qualification or validation should be completed according to local quality requirements and intended use, including any automated platforms.

Conclusion

This comparability study demonstrates that Epoch 2 used with Pyrochrome provides a technically sound replacement for PKFlex with Pyrotell-T under the study conditions evaluated.

Both systems met core analytical requirements, but Epoch 2 delivered stronger consistency in critical performance areas. In addition, Epoch 2 and Pyros express software also support the use of sustainably sourced PyroSmart NextGen recombinant cascade reagent under the same conditions as Pyrochrome. This is considered a safe choice for the future of endotoxin testing.

The findings support customer adoption of Epoch 2 as part of a controlled transition strategy for routine endotoxin testing.

About Technology

Limulus amebocyte lysate (LAL) assays are widely used to detect bacterial endotoxins, which are components of the outer membrane of Gram-negative bacteria. In a kinetic turbidimetric assay, endotoxin activates the LAL cascade and the sample becomes progressively cloudy over time; in a kinetic chromogenic assay, the same biological reaction releases a yellow color signal that is measured over time. Both formats are compendial approaches used for endotoxin testing, but they differ in how the reaction is detected by the instrument.

Epoch 2 reader can be utilized together with other reagents such as recombinant kinetic chromogenic reagent PyroSmart NextGen® to perform endotoxin testing per USP <86>.[5]

As of 25 September 2026, the status of Chapter <86> is expected to change from non-applicable to applicable and for water testing per one of the 7 water monographs from alternative to compendial.

For more information, visit *www.acciusa.com* or contact Technical Services at *techservice@acciusa.com*.

References:

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3. *Pyrotell-T Instructions for use: https://acciusa.com/assets/documents/package-inserts/pyrotell-t-en.pdf*
4. *Pyrochrome Instructions for use: https://acciusa.com/assets/documents/package-inserts/pyrochrome-en.pdf*
5. *Current USP <86> Bacterial Endotoxins Test using Recombinant Reagents*