Use of Endotoxin Detection Methods to Demonstrate Equivalency of a New 96-well Microplate Reader

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charles river

1 Introduction

When onboarding new or upgrading quality control testing instruments, it is essential to ensure equivalency data is generated to compare against the existing method or system. The BioTek ELx808 96-well microplate reader has been discontinued and the Endosafe® PRS 3 Microplate Reader was selected as the replacement. A study was executed to demonstrate that the PRS 3 is equivalent to the existing ELx808 in the performance of bacterial endotoxin detection testing, when used with Charles River test reagents. Equivalency was defined as the ability of the instruments to meet the bacterial endotoxin testing (BET) criteria for each assay performed.

Equivalency Experimental Design:

- Compare three (3) BioTek ELx808 instruments to three (3) Endosafe™ PRS 3 instruments for all tests
- Assays were performed by three (3) analysts
- Assays were performed using four (4) different reagents:
  - Endpoint chromogenic assay (Endochrome™)
  - Kinetic turbidimetric assay (KTA) (Endosafe KTA^{2}™)
  - Kinetic chromogenic assay (KCA) (Endosafe Endochrome-K™)
  - Recombinant cascade chromogenic assay (rCR) (Endosafe Trillium™ Recombinant Cascade Reagent)

Assay Acceptance Criteria:

- Linearity of the standard curve must have a correlation coefficient with an absolute value greater than or equal to 0.980
- The negative control reaction times were greater than the individual reaction times for lambda
- The % CV between endotoxin standard replicates is less than 10%
- The % CV between sample replicates is less than 10%
- Test articles were within ± 2-fold of the concentration

2 Technology

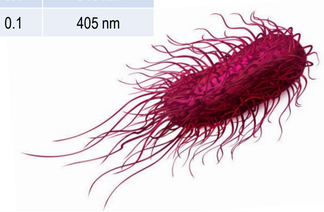
The major differences between the BioTek ELx808 and Endosafe™ PRS 3:

Feature	BioTek ELx808	Endosafe™ PRS 3
Light Source	Halogen Bulb	Xenon flash lamp
Wavelength Selection	Filters	Monochromator
Wavelength Range	340-900 nm	200-999 nm
Loading Method	Top-load	Front-load
Shake Mode	Three speed linear - intensity (set to medium)	Single linear intensity setting
Read Mode	Sweep mode (8 wells at a time)	Scan mode (one well at a time)
Read Interval	30 seconds	60 seconds

3 Methods

- Test articles for each of the four assay types were prepared using Reference Standard Endotoxin (RSE) to provide a common analyte.
- Test articles and standard curves were plated in triplicate on 96-well microplates for all assays tested.
- Each assay type was set up following manufacturer's directions per package inserts.

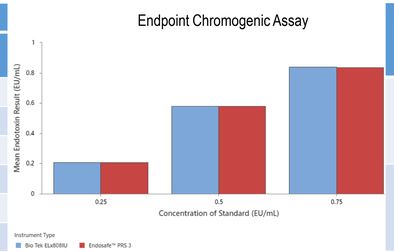
Assay	RSE Test Articles (EU/mL)	Standard Curve (EU/mL)	Lysate Rehydration Volume (mL)	OD	Absorbance
Endpoint Chromogenic	0.75, 0.5, 0.25	1.2 – 0.15	1.4	0.1	405 nm
KCA	1.0, 0.5, 0.01	5.0 – 0.005	3.2	0.1	405 nm
KTA	1.0, 0.5, 0.1	5.0 – 0.05	5.2	0.1	340 nm
Trillium	1.0, 0.5, 0.01	5.0 – 0.005	1.7	0.1	405 nm



4 Results

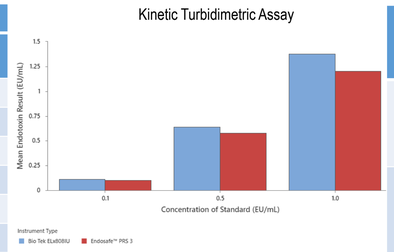
Endpoint Chromogenic Assay

Instrument Type	Serial Number	Average Value (EU/mL)		
		0.75 EU/mL (1.5-0.375 EU/mL)	0.5 EU/mL (1.0-0.25 EU/mL)	0.25 EU/mL (0.5-0.125 EU/mL)
BioTek ELx808	2107291B	0.8616	0.5897	0.2105
	21100427	0.8305	0.5788	0.2074
	21101114	0.8262	0.5745	0.2057
Endosafe™ PRS 3	22102629	0.8609	0.5884	0.2141
	22021801	0.8251	0.5748	0.2050
	22102622	0.8229	0.5736	0.2052



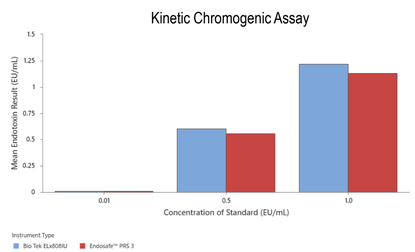
Kinetic Turbidimetric Assay

Instrument Type	Serial Number	Average Value (EU/mL)		
		1.0 EU/mL (2.0-0.5 EU/mL)	0.5 EU/mL (1.0-0.25 EU/mL)	0.1 EU/mL (0.2-0.05 EU/mL)
BioTek ELx808	2107291B	1.2556	0.6196	0.1147
	21100427	1.2897	0.6196	0.1231
	21101114	1.6418	0.7066	0.1126
Endosafe™ PRS 3	22102629	1.2353	0.6232	0.1132
	22021801	1.2494	0.5823	0.1080
	22102622	1.1729	0.5682	0.1007



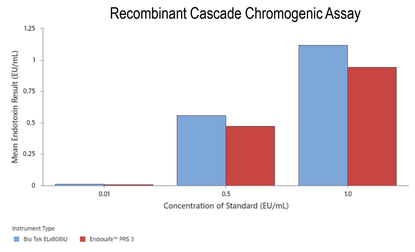
Kinetic Chromogenic Assay

Instrument Type	Serial Number	Average Value (EU/mL)		
		1.0 EU/mL (2.0-0.5EU/mL)	0.5 EU/mL (1.0-0.25 EU/mL)	0.01 EU/mL (0.02-0.005 EU/mL)
BioTek ELx808	2107291B	1.2417	0.6059	0.0087
	21100427	1.2171	0.6103	0.0092
	21101114	1.2011	0.5928	0.0077
Endosafe™ PRS 3	22102629	1.1765	0.5720	0.0086
	22021801	1.1212	0.5658	0.0093
	22102622	1.0996	0.5431	0.0080



Recombinant Cascade Chromogenic Assay

Instrument Type	Serial Number	Average Value (EU/mL)		
		1.0 EU/mL (2.0-0.5EU/mL)	0.5 EU/mL (1.0-0.25 EU/mL)	0.01 EU/mL (0.02-0.005 EU/mL)
BioTek ELx808	2107291B	1.2354	0.5734	0.0118
	21100427	1.0934	0.5340	0.0095
	21101114	1.0181	0.5692	0.0113
Endosafe™ PRS 3	22102629	1.0015	0.4845	0.0109
	22021801	0.9361	0.4341	0.0082
	22102622	0.8850	0.5012	0.0098



5 Conclusion

All of the acceptance criteria to demonstrate equivalency between the BioTek ELx808 and Endosafe™ PRS 3 were met. The tables below show that the lowest correlation coefficient for both instruments was greater than the required absolute value of 0.980. Also shown is that the maximum percent CV for both endotoxin standards and samples was less than 10%. With all the acceptance criteria met the Endosafe™ PRS 3 was shown to be equivalent to the BioTek ELx808.

Correlation Coefficient ≥ |0.980|		
Descriptive Statistics	BioTek ELx808	Endosafe™ PRS 3
Minimum	0.9832	0.9828
Maximum	0.9998	0.9998
Average	0.9940	0.9944

Endotoxin Standard and Sample Replicates % CV < 10%			
Test Article	Descriptive Statistics	BioTek ELx808	Endosafe™ PRS 3
Endotoxin Standard	Maximum	2.38	2.84
	Average	0.80	0.96
Sample	Maximum	5.85	7.15
	Average	1.44	1.59