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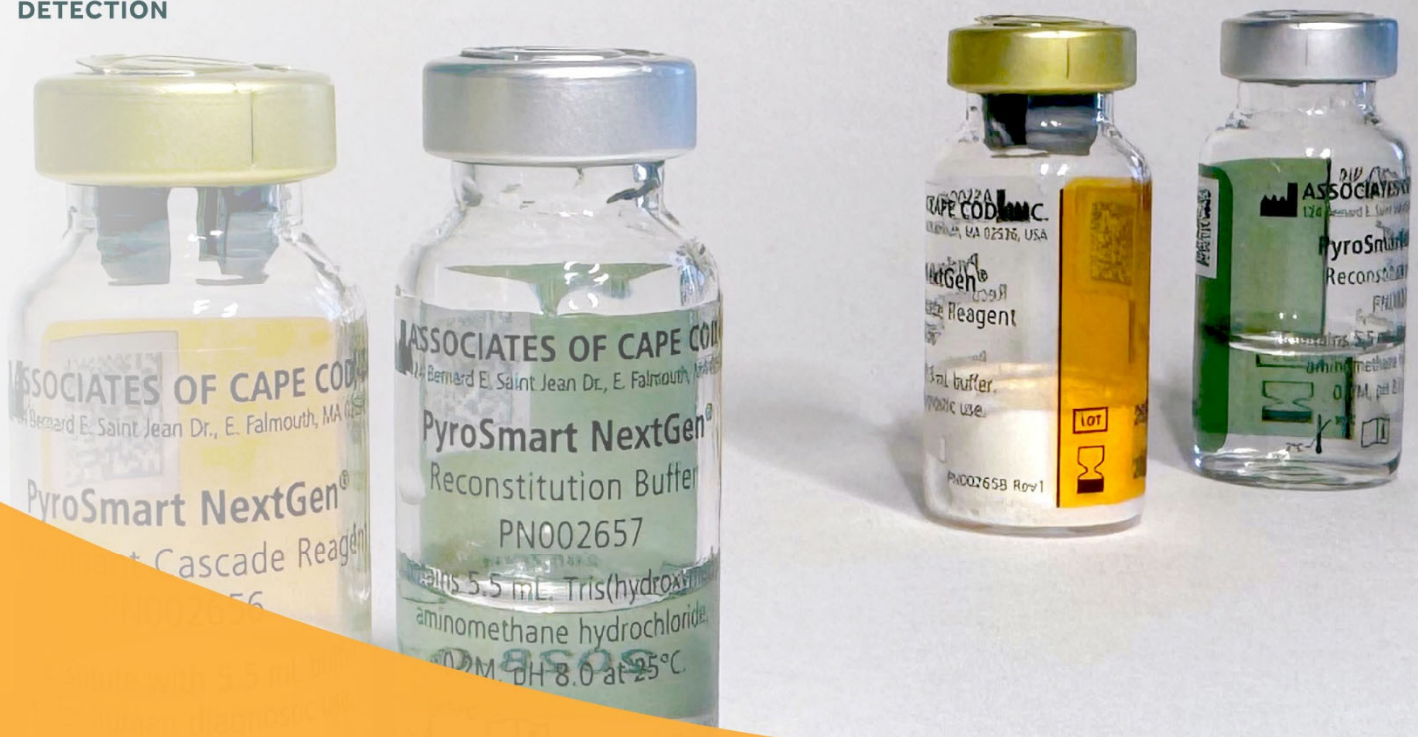

PyroSmart®

NEXTGEN Recombinant Cascade Reagent (rCR)

Primary Validation Package

Associates of Cape Cod, Inc. - a Seikagaku Group Company
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CORP_0920

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PyroSmart NextGen®

Primary Validation Package

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PyroSmart NextGen®

Primary Validation Package

Background

The PyroSmart NextGen® Recombinant Cascade Reagent Kit (PSNG rCR Kit) provides a sustainable alternative to Limulus Amebocyte Lysate (LAL) reagent for the Bacterial Endotoxins Test (BET). The PSNG rCR is a recombinant cascade reagent as defined and described in USP <86> (1).

The PSNG rCR kit can be used for a wide variety of endotoxin tests, ranging from water testing to release and stability testing of therapeutics such as injectable drugs and biologics. The product employs recombinant proteins to carry out the same LAL clotting reaction cascade as traditional LAL reagents, to cleave a chromogenic substrate whose detection facilitates the quantitation of endotoxin present in a sample. For the reaction, Factor C becomes activated in the presence of endotoxin, leading to the activation of Factor B, which stimulates the activity of the Proclotting (PC) Enzyme, resulting in the proteolytic cleavage of the colorless chromogenic substrate (Figure 1). This liberates paranitroaniline (pNA), a yellow substance that absorbs at 405nm, whose quantity reflects the amount of endotoxin in the sample, as assessed using a standard curve. The assay can be performed using the same validated absorbance readers and software platforms as employed for other kinetic photometric assays per USP <85> (2).

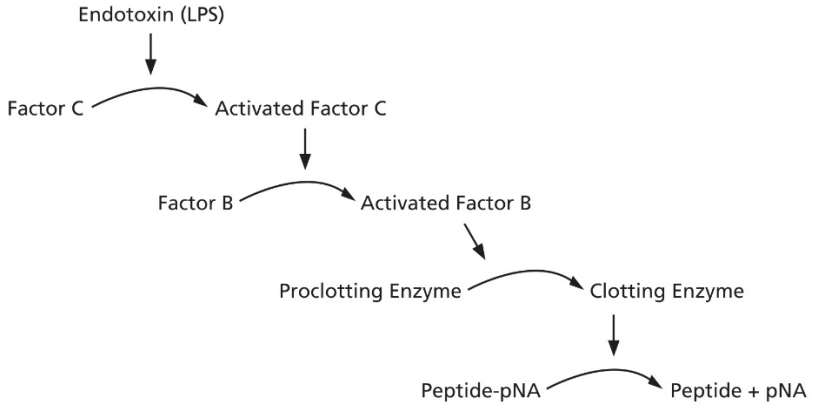

Figure 1: The cascade mechanism starting with endotoxin-activation of Factor C and yielding absorbance increases as a result of pNA release.

The kinetic chromogenic assay can be performed either in 96-well microplates as a rate assay or an onset time assay, or as an onset time assay in a tube reader. This primary validation package describes the use of a microplate reader. The software settings are summarized in the test method description (Table 2). The assay relies on a standard curve generated using a series of standard concentrations, which are commercially available endotoxin preparations of known activity. The standard series serves two purposes: as an internal control to establish the validity of the assay, and to calculate endotoxin concentrations in test samples.

Intended Use

The PSNG rCR is used for the detection and quantification of bacterial endotoxins, as described in USP <86> (1). When used according to U.S. Food and Drug Administration (FDA) guidelines (3), it may be applied for release testing of human injectable drugs (including biological products), animal injectable drugs, and medical devices.

The PSNG rCR may also be used for the quantitation of endotoxin in non-compendial articles (e.g. raw materials, including water, and for in-process monitoring of endotoxin levels). Local regulatory guidelines should be followed when implementing the PSNG rCR.

The PSNG rCR is not intended for use in the detection of endotoxin in clinical samples for the diagnosis of human disease such as endotoxemia in humans.

PyroSmart NextGen®

Primary Validation Package

Method Validation

The PSNG rCR is used for the detection and quantification of bacterial endotoxins and is therefore categorized as a quantitative test for impurities. PSNG rCR was evaluated for performance and analytical characteristics using specifications for linearity, accuracy, precision, range, quantitation limit, specificity and robustness as required by the ICH Q2 guideline (4) and USP <1225> (5). The data evaluated for each parameter includes a maximum of 48 standard curves (24 rate assay, 24 onset assay) using two kits over multiple days by four analysts. All results satisfy the pre-defined acceptance criteria.

Materials and Equipment

Table 1. Method Validation Materials and Equipment

Reagents	**Part Number/Lot Number**
PSNG rCR	ACC PN002630, Lot number 2630001 and 2630003*
PSNG rCR Reconstitution Buffer	ACC PN002631, Lot number 2631001
LAL Reagent Water (LRW)	ACC WP050C, Lot number 314-4327 and 314-4319
United States Pharmacopeia Reference Standard Endotoxin	Lot Number H0K254
Glucan Standard	ACC PN001189, Lot number 1189041 and 1189047
Equipment	**ID**
Microplate Reader	Multiple Agilent BioTek ELx808 absorbance readers (calibrated)
Pipettes	100µL Manual (calibrated) 200µL Manual (calibrated) 1000µL Manual (calibrated) 5000µL Manual (calibrated) Eppendorf Combitip Repeater (calibrated)
Vortex Mixer	N/A (calibrated)
Timer	N/A (calibrated)
Materials	**Part Number**
Dilution Tubes	ACC TB240-5 (12x75) ACC TB013-5 (13x100)
Pipette Tips	ACC PPT25 (250µL) ACC PPT10 (1000µL) Eppendorf 5000µL 2.5mL Eppendorf Combitips
Pyroplate®	ACC CA961-10

*This primary validation package uses PNG050 as the representative reagent to demonstrate suitability for intended use. Performance equivalency of PNG100 to PNG050 was confirmed under its design verification.

Assay Conditions and Data Interpretation

The PSNG rCR can be used to quantify endotoxin concentration in two ways:

Onset Time Assay: where the time taken to reach a threshold OD (referred to as onset time) is determined. Higher endotoxin concentrations result in shorter onset times. The standard curve is constructed by plotting the log onset time (Y-axis) against the log standard concentration (X-axis) and is used to calculate endotoxin concentrations in samples.

Rate Assay: where the mean rate (Vmean: mAbs/min) is calculated over the course of the test. Higher endotoxin concentrations result in higher Vmean values. The standard curve is constructed by plotting Vmean (Y-axis) against the standard concentration (X-axis) and is used to determine endotoxin concentrations in samples.

The software settings for both types are summarized in Table 2.

Table 2. Microplate Reader Software Settings for PSNG rCR Assays

	Onset Time Assay	**Rate Assay**
Shake	10 sec	10 sec
Read	Kinetic, Absorbance	Kinetic, Absorbance
Wavelength	405nm	405/490nm*
Reading Interval	30 sec**	30 sec**
Incubation Time	60 min***	60 min***
Data Reduction	Onset OD = 0.03 or Threshold mOD = 30	Pyros® eXpress: Vmean Gen5®: MeanV Softmax® Pro: Vmax

*Or 405/492nm depending on the ability of the plate reader
**Interval may vary based on plate reader
***Incubation time may vary under actual conditions of use

Endotoxin concentrations of all test samples (including standards and controls) are calculated by interpolation against the standard curve using the equation for a straight line (linear regression) as follows:

Onset Time Assay:
Log Endotoxin Concentration = (Log Onset Time – Y-intercept) / Slope

Rate Assay (applies to plate reader method only):
Endotoxin Concentration = (Vmean – Y-intercept) / Slope

Polynomial regression can be used for Onset Time assays.

Additional details regarding the assay procedure can be found in the PSNG rCR Instructions for Use (6).

Outliers

Outlier removal was performed per the Rosner's outlier test. All assays were valid after removal of outliers and final analysis of data was performed.

Validity Criteria

Table 3. Validity Criteria

Parameter	Validity Criteria
Negative control	Onset time assay: the onset time must be greater than that of the lowest standard concentration. Rate assay: the Vmean (MeanV or Vmax) must be lower than that of the lowest standard concentration. It should be less than or equal to 1.0 mAbs/min.
Standard curve	Correlation coefficient with an absolute value of ≥ 0.980
Positive product controls (PPC)	PPC recovery % must be within 50 – 200% of the nominal concentration of the added endotoxin

Linearity

3 Days, 4 Analysts, 2 Kits, 24 Rate Assays, 24 Onset Time Assays

Linearity is the ability of an analytical procedure (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample. Data from 48 standard curves (24 rate assay, 24 onset time assay) using two kits over three days by four analysts was analyzed for regression to determine linearity.

Table 4. Rate Assay Linearity Data

Plate ID	Standard Curve			Linearity Acceptance Criteria
	Slope	Y-int	R	
R-1	33.7	0.105	0.999	**≥0.980**
R-2	36.6	0.073	0.999	
R-3	35.5	0.011	1.000	
R-4	39.5	0.062	1.000	
R-5	36.2	0.031	0.999	
R-6	33.8	0.072	0.999	
R-7	42.3	0.130	1.000	
R-8	46.6	0.077	1.000	
R-9	45.0	0.060	1.000	
R-10	35.1	0.085	1.000	
R-11	37.4	0.083	1.000	
R-12	37.1	0.078	1.000	
R-13	35.2	0.106	1.000	
R-14	37.6	0.079	1.000	
R-15	38.1	0.027	1.000	
R-16	38.0	0.074	1.000	
R-17	38.2	0.066	1.000	
R-18	34.7	0.079	0.998	
R-19	39.2	0.134	1.000	
R-20	43.6	0.114	1.000	
R-21	38.9	0.077	1.000	
R-22	37.7	0.103	1.000	
R-23	40.2	0.032	0.999	
R-24	39.1	0.083	1.000	

Table 5. Onset Time Assay Linearity Data

Plate ID	Standard Curve			Linearity Acceptance Criteria
	Slope	Y-int	R	
O-1	-0.239	2.85	0.997	**≥0.980**
O-2	-0.241	2.84	0.997	
O-3	-0.241	2.85	0.998	
O-4	-0.244	2.83	0.999	
O-5	-0.240	2.84	0.998	
O-6	-0.236	2.86	0.997	
O-7	-0.246	2.85	0.999	
O-8	-0.249	2.82	0.998	
O-9	-0.241	2.84	0.998	
O-10	-0.224	2.87	0.998	
O-11	-0.233	2.86	0.998	
O-12	-0.238	2.85	0.997	
O-13	-0.238	2.85	0.998	
O-14	-0.225	2.83	0.996	
O-15	-0.244	2.84	0.998	
O-16	-0.240	2.85	0.998	
O-17	-0.237	2.85	0.998	
O-18	-0.238	2.85	0.998	
O-19	-0.246	2.84	0.998	
O-20	-0.243	2.84	0.998	
O-21	-0.248	2.83	0.996	
O-22	-0.229	2.85	0.998	
O-23	-0.242	2.83	0.998	
O-24	-0.244	2.82	0.998	

Table 6. Linearity Summary

	Linearity		
Assay	Minimum	Maximum	Criteria
Rate	0.998	1.000	≥0.980
Onset Time	0.996	0.999	≥0.980

Accuracy

3 Days, 4 Analysts, 2 Kits, 24 Rate Assays, 24 Onset Time Assays

Accuracy is the closeness of test results obtained by an analytical procedure to a value, which is either a conventional true value or an accepted reference value. Data from 48 standard curves (24 rate assay, 24 onset time assay) using two kits over three days by four analysts was analyzed for endotoxin recovery of the nominal endotoxin concentration to determine accuracy.

Table 7. Rate Assay Accuracy Data

Plate ID	Accuracy (%)					Accuracy Acceptance Criteria
	0.006	0.0125	0.025	0.05	0.1	
R-1	105	109	98	96	101	**50-200%**
R-2	107	107	101	94	101	
R-3	122	103	94	98	101	
R-4	120	100	96	98	101	

R-5	118	103	98	95	101	
R-6	125	103	97	95	101	
R-7	98	99	103	99	100	
R-8	109	101	98	99	100	
R-9	102	103	100	98	100	
R-10	113	100	99	97	101	
R-11	97	107	97	100	100	
R-12	114	102	99	97	101	
R-13	102	102	102	98	100	
R-14	96	103	100	100	100	
R-15	116	104	96	98	101	
R-16	118	100	98	97	101	
R-17	118	104	96	97	101	
R-18	122	102	102	93	102	
R-19	84	99	105	101	100	
R-20	102	100	102	98	100	
R-21	100	100	100	100	100	
R-22	106	98	102	97	100	
R-23	121	102	100	94	101	
R-24	107	103	98	99	100	

Table 8. Onset Time Assay Accuracy Data

Plate ID	Accuracy (%)					Accuracy Acceptance Criteria
	0.005	**0.05**	**0.5**	**5.0**	**50**	
O-1	86	107	129	113	81	**50-200%**
O-2	75	115	140	113	75	
O-3	80	112	126	110	81	
O-4	86	114	113	106	89	
O-5	79	112	132	109	80	
O-6	78	116	131	106	82	
O-7	84	132	93	109	92	
O-8	79	118	120	111	81	
O-9	79	116	126	112	80	
O-10	77	119	119	114	81	
O-11	77	119	124	111	80	
O-12	77	116	129	114	77	
O-13	84	107	127	114	80	
O-14	Outlier	87	121	116	86	
O-15	83	109	127	111	81	
O-16	82	116	114	117	81	
O-17	82	122	113	107	87	
O-18	85	111	123	107	84	
O-19	88	138	75	117	95	
O-20	77	115	120	118	79	
O-21	71	129	134	108	77	
O-22	82	115	117	112	83	
O-23	82	110	125	111	81	
O-24	82	109	124	116	80	

Table 9. Accuracy Summary

Accuracy				
Assay	**Standard (EU/mL)**	**Minimum**	**Maximum**	**Criteria**
Rate	0.00625	84%	125%	**50-200%**
	0.0125	98%	109%	
	0.025	94%	105%	
	0.05	93%	101%	
	0.10	100%	102%	
Onset Time	0.005	71%	88%	**50-200%**
	0.05	87%	138%	
	0.5	75%	140%	
	5.0	106%	118%	
	50	75%	95%	

Repeatability (CV%)

3 Days, 3 Analysts, 2 Kits, 18 Rate Assays, 18 Onset Time Assays

Repeatability shows the agreement between a series of measurements obtained under the same operating conditions (same test) over a short interval of time (same plate). Data from 36 standard curves (18 rate assay, 18 onset time assay) using two kits over three days by three analysts was analyzed for concentration CV% to show repeatability.

Table 10. Rate Assay Repeatability Data

Plate ID	Concentration CV (%)					Concentration CV (%) Acceptance Criteria
	0.006	**0.0125**	**0.025**	**0.05**	**0.1**	
R-1	21	7	12	6	9	**≤25% 0.00625 EU/mL ≤20% 0.0125-0.10 EU/mL**
R-2	14	10	10	10	9	
R-3	7	9	10	11	11	
R-4	14	11	10	5	7	
R-5	13	5	9	8	4	
R-6	18	8	8	8	7	
R-7	15	9	7	8	10	
R-8	8	6	8	5	3	
R-9	7	7	9	7	6	
R-10	17	13	7	8	18	
R-11	12	16	12	3	8	
R-12	11	10	10	5	6	
R-13	14	10	8	8	8	
R-14	18	11	9	7	9	
R-15	15	12	9	14	10	
R-16	9	12	11	7	7	
R-17	14	9	6	12	5	
R-18	8	8	10	6	5	

Table 11. Onset Time Assay Repeatability Data

Plate ID	Concentration CV (%)					Concentration CV (%) Acceptance Criteria
	0.005	0.05	0.5	5.0	50	
O-1	25	25	10	12	13	**≤35% 0.005 EU/mL ≤30% 0.05-50 EU/mL**
O-2	13	15	12	9	10	
O-3	12	6	7	6	11	
O-4	14	12	21	6	9	
O-5	14	9	9	10	6	
O-6	14	9	13	7	16	
O-7	10	7	8	7	6	
O-8	9	10	9	6	5	
O-9	18	13	15	4	8	
O-10	24	11	12	12	9	
O-11	Outlier	24	11	7	13	
O-12	17	14	14	9	7	
O-13	14	12	12	6	9	
O-14	17	11	23	6	12	
O-15	30	8	11	6	11	
O-16	8	7	8	6	5	
O-17	12	9	10	7	11	
O-18	13	8	8	7	8	

Table 12. Repeatability Summary

Repeatability				
Assay	**Standard (EU/mL)**	**Minimum**	**Maximum**	**Criteria**
Rate	0.00625	7%	21%	**≤25%**
	0.0125	5%	16%	**≤20%**
	0.025	6%	12%	
	0.05	3%	14%	
	0.10	3%	18%	
Onset Time	0.005	8%	30%	**≤35%**
	0.05	6%	25%	**≤30%**
	0.5	7%	23%	
	5.0	4%	12%	
	50	5%	16%	

Intermediate Precision (95% CI for CV%)

3 Days, 3 Analysts, 2 Kits, 18 Rate Assays, 18 Onset Time Assays

Intermediate precision shows agreement between a series of measurements obtained within the laboratory on different days with different analysts and different equipment. Data from 36 standard curves (18 rate assay, 18 onset time assay) using two kits over three days by three analysts was analyzed for the 95% confidence interval of the concentration CV% to show intermediate precision.

Table 13. Rate Assay Intermediate Precision 95% CI of CV Data

STD (EU/mL)	AVE CONC (Outliers Not Removed)	SD	CV	total sample#	95% confidence Interval of variance (upper)*1	95% confidence Interval of variance (lower)*2
0.006	0.006757	0.001136	17	144	15	19

0.0125	0.0128	0.001277	10	144	9	11
0.025	0.02483	0.002231	9	144	8	10
0.05	0.04869	0.003772	8	144	7	9
0.1	0.1006	0.008069	8	144	7	9

Table 14. Onset Time Assay Intermediate Precision 95% CI of CV Data

STD (EU/mL)	AVE CONC (Outliers Not Removed)	SD	CV	total sample#	95% confidence Interval of variance (upper)*1	95% confidence Interval of variance (lower)*2
0.005	0.004049	0.0008797	22	144	20	24
0.05	0.05828	0.007905	14	144	12	15
0.5	0.6047	0.1044	17	144	16	19
5	5.563	0.4484	8	144	7	9
50	41.19	4.559	11	144	10	12

Reproducibility (95% CI for CV%)

3 Days, 4 Analysts, 2 Kits, 24 Rate Assays, 24 Onset Assays

Reproducibility expresses the precision between laboratories. Data from 48 standard curves (24 rate assay, 24 onset time assay) using two kits over three days by four analysts was analyzed for both the concentration CV% and the 95% confidence interval of the concentration CV% to show reproducibility.

Table 15. Rate Assay Reproducibility Data

Standard (EU/mL)	Avg Conc (Outliers Not Removed)	SD	CV (%)	Sample #	Minimum %CHI	Maximum %CHI	Criteria
0.00625	0.006797	0.001081	16	192	15	17	**≤25% 0.00625 EU/mL ≤20% 0.0125 – 0.10 EU/mL**
0.0125	0.01278	0.001264	10	192	9	11	
0.025	0.02481	0.002227	9	192	8	10	
0.05	0.04869	0.003579	7	192	7	8	
0.1	0.1006	0.007495	7	192	7	8	

Table 16. Onset Time Assay Reproducibility Data

Standard (EU/mL)	Avg Conc (Outliers Not Removed)	SD	CV (%)	Sample #	Minimum %CHI	Maximum %CHI	Criteria
0.005	0.004047	0.0008141	20	192	19	22	**≤35% 0.005 EU/mL ≤30% 0.05-50 EU/mL**
0.05	0.05801	0.0076	13	192	12	14	
0.5	0.6071	0.09657	16	192	15	17	
5.0	5.583	0.4352	8	192	7	9	
50	40.95	4.312	11	192	10	12	

Range

3 Days, 4 Analysts, 2 Kits, 24 Rate Assays, 24 Onset Time Assays

The range of an analytical procedure defines the interval between the upper and lower levels of analyte (including these levels) for which it has been demonstrated that the procedure delivers results with suitable precision, accuracy. and linearity. Data from 48 standard curves (24 rate, 24 onset time assay) using two kits over three days by four analysts was analyzed for reproducibility, repeatability, accuracy, and linearity to show range.

Table 17. Rate Assay Range Data

Analysis Method	Minimum	Maximum	Criteria
Reproducibility (95% CI of CV%)	7%	17%	≤25% 0.00625 EU/mL ≤20% 0.0125 – 0.10 EU/mL
Reproducibility (CV%)	7%	16%	
Repeatability	3%	21%	
Accuracy	84%	125%	50-200%
Linearity	0.998	1.000	≥0.980

Table 18. Onset Time Assay Range Data

Analysis Method	Minimum	Maximum	Criteria
Reproducibility (95% CI of CV%)	7%	22%	≤35% 0.005 EU/mL ≤30% 0.05-50 EU/mL
Reproducibility (CV%)	8%	20%	
Repeatability	4%	30%	
Accuracy	71%	140%	50-200%
Linearity	0.996	0.999	≥0.980

Quantitation Limit

3 Days, 4 Analysts, 2 Kits, 24 Onset Time Assays

The quantitation limit is the lowest concentration of analyte in a sample that can be quantitatively determined with acceptable precision and accuracy. Data from 24 onset time assay standard curves using two kits over three days by four analysts was analyzed for concentration CV% at 0.005 EU/mL, accuracy at 0.005 EU/mL, and linearity.

Table 19. Onset Time Assay Limit of Quantitation Summary

Analysis Method	Minimum	Maximum	Criteria
0.005 EU/mL Concentration %CV	6%	30%	≤35%
0.005 EU/mL Accuracy	71%	88%	50-200%
Linearity	0.996	0.999	≥0.980

Specificity (Reactivity with Beta Glucan)

2 Days, 1 Analyst, 2 Kits, 8 Rate Assays, 8 Onset Time Assays

Specificity is the ability of an analytical procedure to assess the analyte (endotoxin) unequivocally in the presence of other components that may be expected to be present in the sample matrix, such as (1→3)-beta-D-glucan. Data from 16 standard curves with and without glucan added (8 rate assay, 8 onset time

assay) using two kits over two days by one analyst was analyzed using linear regression. The 95% CI and Pearson's r statistical test of the linear regression slope were performed to show specificity.

Table 20. Specificity Rate Assay Data

Plate ID	Glucan	Standard Curve			Linearity Acceptance Criteria
		Slope	Y-int	R	
R-31	W	42.5	-0.005	0.999	≥0.980
	W/O	41.6	0.024	0.999	
R-32	W	42.7	0.010	0.999	
	W/O	40.1	0.063	1.000	
R-33	W	41.7	0.024	0.999	
	W/O	39.7	0.065	1.000	
R-34	W	41.9	-0.008	0.998	
	W/O	41.1	0.007	0.999	

Table 21. Specificity Onset Time Assay Data

Plate ID	Glucan	Standard Curve			Linearity Acceptance Criteria
		Slope	Y-int	R	
ON-25	W	-0.255	2.81	0.998	≥0.980
	W/O	-0.240	2.83	0.999	
ON-26	W	-0.258	2.80	0.998	
	W/O	-0.245	2.84	0.998	
ON-27	W	-0.250	2.82	0.997	
	W/O	-0.242	2.85	0.999	
ON-28	W	-0.261	2.81	0.999	
	W/O	-0.242	2.84	0.998	

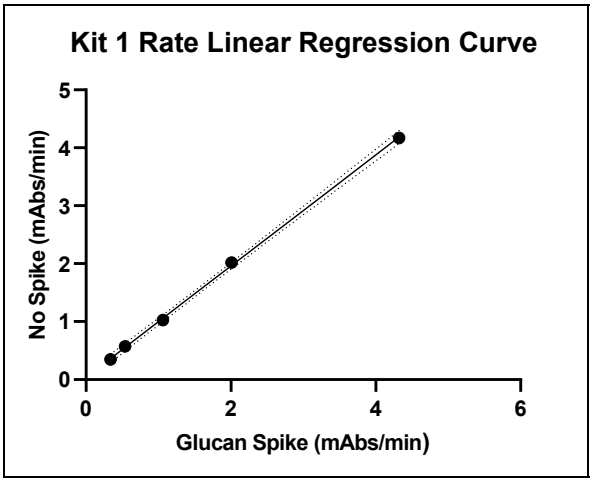

Figure 2. Kit 1 Rate Linear Regression

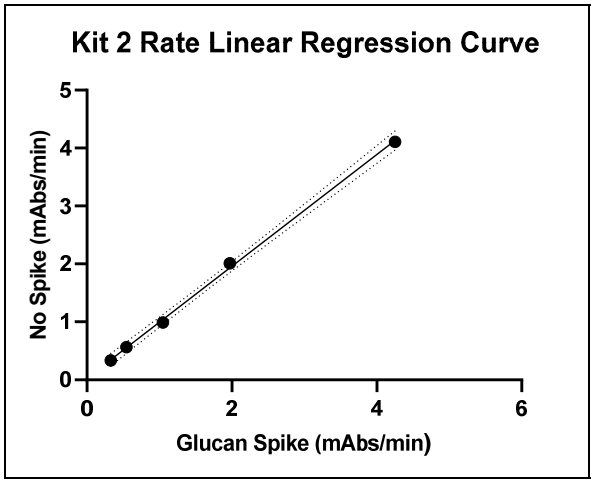

Figure 3. Kit 2 Rate Linear Regression

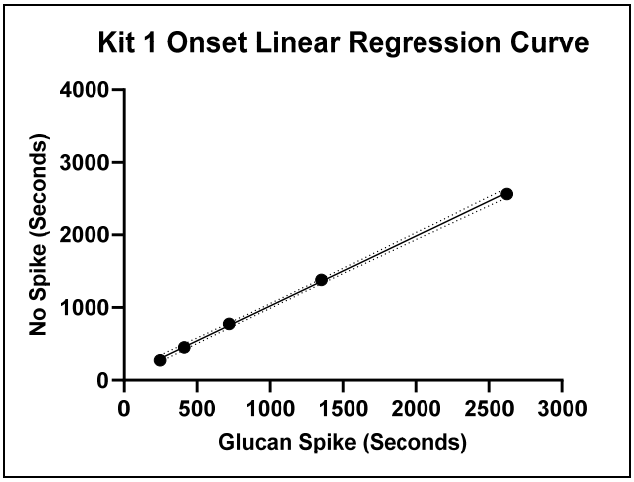

Figure 4. Kit 1 Onset Time Linear Regression

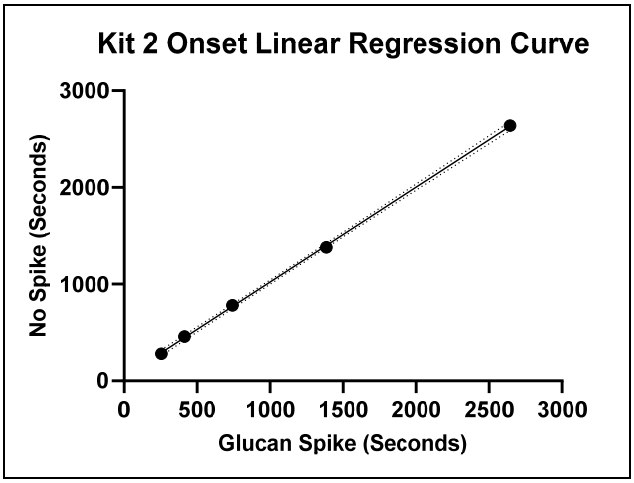

Figure 5. Kit 2 Onset Time Linear Regression

Table 22. Specificity Rate and Onset Time Assay Regression Analysis

Analysis	Rate Assay		Onset Time Assay	
	Kit 1	Kit 2	Kit 1	Kit 2
R square	0.9996	0.9990	0.9995	0.9998
P value	<0.0001	<0.0001	<0.0001	<0.0001
Is Slope Significantly Non-Zero?	Yes	Yes	Yes	Yes

Table 23. Specificity Summary

	Kit 1 Rate	Kit 2 Rate	Kit 1 Onset Time	Kit 2 Onset Time
95% Confidence Intervals				
Slope	0.9211 to 0.9949	0.9092 to 1.020	0.9235 to 0.9990	0.9554 to 1.006
Y-intercept	-0.03863 to 0.1241	-0.09009 to 0.1500	9.225 to 113.0	5.923 to 76.02
X-intercept	-0.1335 to 0.03919	-0.1627 to 0.08956	-121.3 to -9.312	-79.13 to -5.922
Is slope significantly non-zero?				
F	6812	3066	6573	15380
DFn, DFd	1, 3	1, 3	1, 3	1, 3
P value	<0.0001	<0.0001	<0.0001	<0.0001
Deviation from zero?	Significant	Significant	Significant	Significant
Equation	Y = 0.9580*X + 0.04273	Y = 0.9647*X + 0.02998	Y = 0.9612*X + 61.09	Y = 0.9805*X + 40.97

Robustness

Multiple Days, 4 Analysts, 2 Kits, 8 Rate Assays, 8 Onset Time Assays

Robustness is a measure of the capacity of an analytical procedure to remain unaffected by small, but deliberate variations in parameters, which indicates the reliability of the method during normal use. Data from 16 standard curves reconstituted for three vs. 20 minutes (8 rate assay, 8 onset time assay) using two kits over multiple days by four analysts was analyzed for linearity, accuracy, and repeatability to show robustness. The Pearson's r statistical test was also performed using the linear regression slope.

Table 24. Robustness Rate Assay 20 Minute Post Reconstitution Data

	Plate ID	Standard Curve		
		Slope	Y-int	R
Kit 1	R-35	35.2	0.090	1.000
	R-36	45.3	0.039	0.999
	R-37	44.5	0.073	0.999
	R-38	41.2	0.103	1.000
Kit 2	R-39	32.5	0.131	1.000
	R-40	38.0	0.221	1.000
	R-41	37.5	0.103	0.999

	R-42	44.1	0.080	1.000

Table 25. Robustness Rate Assay 20 Minute Post Reconstitution Data

	Plate ID	Concentration CV (%)					Accuracy (%)				
		0.006	0.0125	0.025	0.05	0.1	0.006	0.0125	0.025	0.05	0.1
Kit 1	**R-35**	18	19	10	5	11	102	101	98	100	100
	R-36	12	5	14	14	6	120	105	97	96	101
	R-37	11	9	6	6	5	115	107	96	96	101
	R-38	11	16	9	5	6	97	106	100	98	100
Kit 2	**R-39**	24	11	11	5	8	97	104	99	100	100
	R-40	13	19	11	8	5	122	97	99	97	101
	R-41	10	8	6	9	6	102	99	108	94	101
	R-42	6	10	4	9	8	111	97	97	101	100

Table 26. Robustness Rate Assay Summary

Analysis Method	Kit 1		Kit 2		Criteria
	Minimum	Maximum	Minimum	Maximum	
Concentration %CV	5%	19%	4%	24%	≤25% 0.00625 EU/mL ≤20% 0.0125-0.10 EU/mL
Accuracy	96%	120%	94%	122%	50-200%
Linearity	0.999	1.000	0.999	1.000	≥0.980

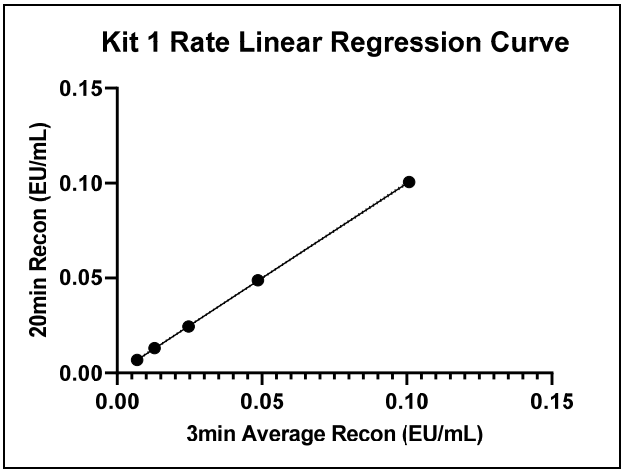

Figure 6. Kit 1 Rate Robustness Linear Regression

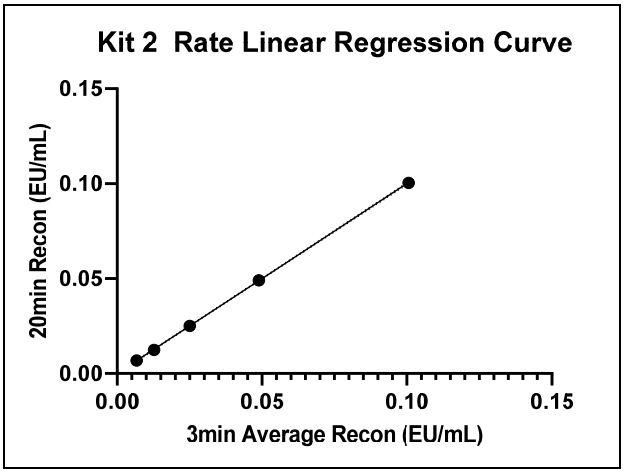

Figure 7. Kit 2 Rate Robustness Linear Regression

Table 27. Robustness Rate Assay Regression Analysis Summary

Analysis Method	Kit 1	Kit 2	Criteria
Slope	0.9995	1.001	>0.90
R square	1.000	1.000	N/A
P value	<0.0001	<0.0001	N/A
Deviation from Zero	Significant	Significant	Significant

Table 28. Robustness Onset Time Assay 20 Minute Post Reconstitution Data

	Plate ID	Standard Curve		
		Slope	Y-int	R
Kit 1	**O-29**	-0.238	2.83	0.998
	O-30	-0.243	2.83	0.999
	O-31	-0.241	2.83	0.999
	O-32	-0.242	2.82	0.998
Kit2	**O-33**	-0.237	2.84	0.998
	O-34	-0.237	2.83	0.999
	O-35	-0.239	2.83	0.997
	O-36	-0.237	2.82	0.998

Table 29. Robustness Onset Time Assay 20 Minute Post Reconstitution Data

	Plate ID	Concentration CV (%)					Accuracy (%)				
		0.005	0.05	0.5	5.0	50	0.005	0.05	0.5	5.0	50
Kit 1	**O-29**	25	9	11	10	11	86	105	127	112	81
	O-30	18	16	16	6	9	86	117	109	107	89
	O-31	11	10	9	8	11	83	112	123	103	87
	O-32	18	7	14	6	12	83	124	98	124	83
Kit 2	**O-33**	19	18	13	10	14	88	101	130	109	84
	O-34	18	9	11	6	6	91	107	111	107	90
	O-35	18	10	6	8	14	78	115	131	110	79
	O-36	22	13	16	3	15	90	113	94	125	86

Table 30. Robustness Onset Time Assay Summary

Analysis Method	Kit 1		Kit 2		Criteria
	Minimum	Maximum	Minimum	Maximum	
Concentration %CV	6%	25%	3%	22%	≤35%
Accuracy	81%	127%	78%	131%	50-200%
Linearity	0.998	0.999	0.997	0.999	≥0.980

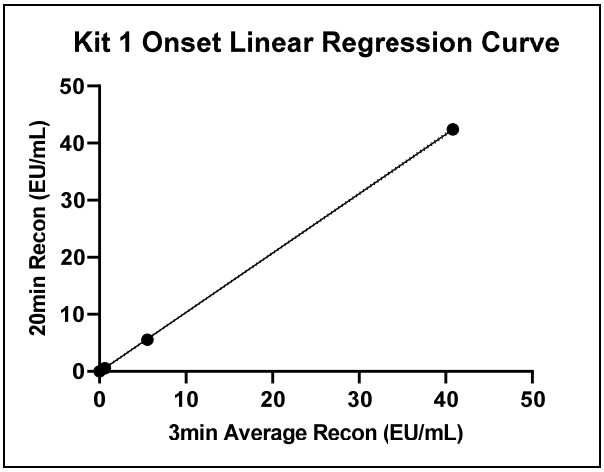

Figure 8. Kit 1 Onset Time Robustness Linear Regression

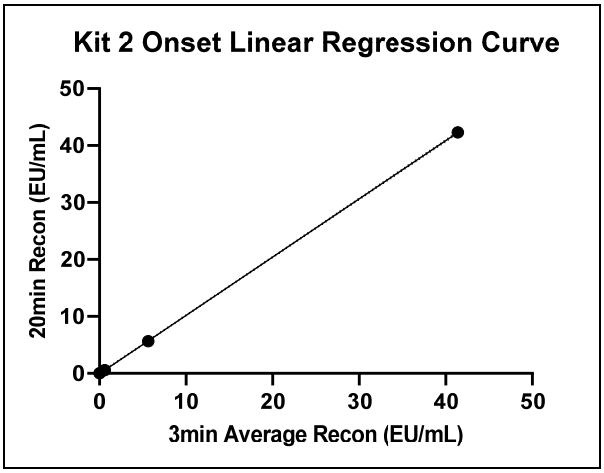

Figure 9. Kit 2 Onset Time Robustness Linear Regression

Table 31. Robustness Onset Time Assay Regression Analysis Summary

Analysis Method	Kit 1	Kit 2	Criteria
Slope	1.040	1.023	>0.90
R square	1.000	1.000	N/A
P value	<0.0001	<0.0001	N/A
Deviation from Zero	Significant	Significant	Significant

Conclusion

The PyroSmart NextGen® rCR was evaluated for performance and analytical characteristics using linearity, accuracy, precision, range, quantitation limit, specificity and robustness as is required by the ICH Q2 guideline and USP <1225>. The results support its use for the detection and quantification of bacterial endotoxins as described in USP <86>.

FDA Type V Master File

Given that the product will be used by FDA regulated manufacturers for assessing sterility assurance, a Type V Master File format was selected as the format to submit the relevant information to the US FDA.

A Type V Master File contains administrative information (e.g. the department certification) as well as confidential proprietary quality information on the raw materials formulation manufacturing including batch records, product control, specifications, analytical methods, reference standards, container closures and stability data.

The PSNG rCR kit is manufactured in compliance with current GMP regulations.

Should access to the PSNG MF be required, contact Technical Services at techservice@acciusa.com.

References

1. Bacterial Endotoxins Test Using Recombinant Reagents <86>, United States Pharmacopeia (current revision), United States Pharmacopeial Convention, Rockville, MD.

2. Bacterial Endotoxins Test <85>, United States Pharmacopeia (current revision), United States Pharmacopeial Convention, Rockville, MD.

3. Guidance for Industry, Pyrogen and Endotoxins Testing: Questions and Answers. U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration, July 2012.

4. Validation of Analytical Procedures Q2 (R1), International Council for Harmonisation (ICH), Geneva, Switzerland.

5. Validation of Compendial Procedures <1225>, United States Pharmacopeia (current revision), United States Pharmacopeial Convention, Rockville, MD.

6. PyroSmart NextGen® Recombinant Cascade Reagent Instructions For Use (current revision).