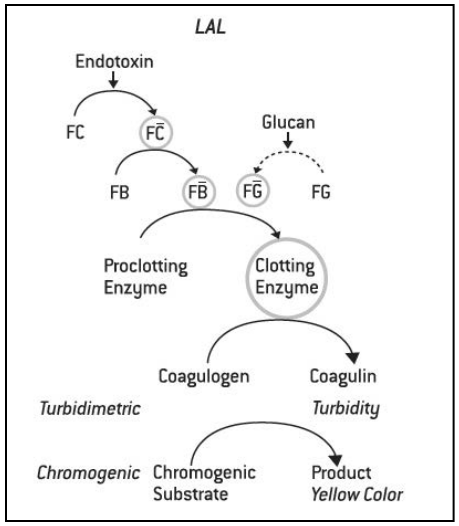
The recombinant Factor C Alternative Endotoxin Method

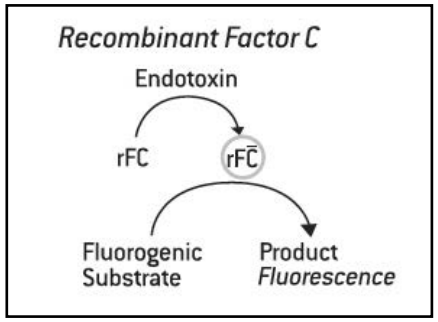
Primed for the Big Time

Agenda

- What is rFC?
- Why implement rFC?
- Overview
- Lilly Validation / Equipment Strategy
- Data
  - Assay Performance
  - Validation
  - Comparability
- Summary

What is rFC?

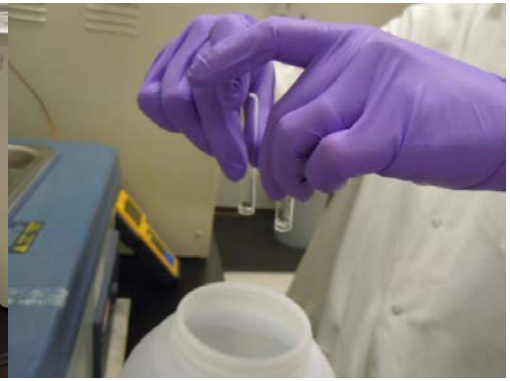




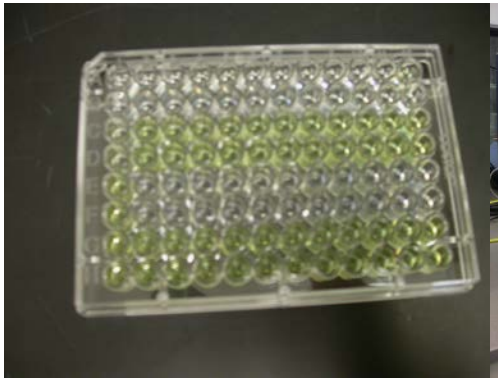
- recombinant Factor C (rFC) is a recombinantly manufactured protein used for the detection of bacterial endotoxins
- Factor C activation is the first step in the Horseshoe crab clotting cascade and bypasses the factor G glucan false positive pathway
- End-point fluorescence is a technique to detect activation of the Factor C pathway
- Bacterial endotoxins detection by end-point fluorescence using rFC is a non-compendia alternative endotoxin method
  - The data presented herein supports the promotion of end-point fluorescence/rFC into the compendia bacterial endotoxins chapters

An Evolution of Pyrogen & Endotoxin Testing at Lilly

Rabbit Pyrogen Testing – Up to the mid 1980s



Gel Clot Testing – mid 1980s to mid-1990s



Kinetic Photometric Testing – mid 1990s to present

rFC – Present to the Future

Why rFC?

- Our experience and data to date indicate that the use of rFC in the BET method is equivalent or superior to using LAL
- The method removes reliance on an animal-sourced reagent, consistent with the 3 R principle: Replacement, Reduction, Refinement
- The method validation is relatively easy compared to other available microbiological alternative methods
  - Calculation values and units do not change

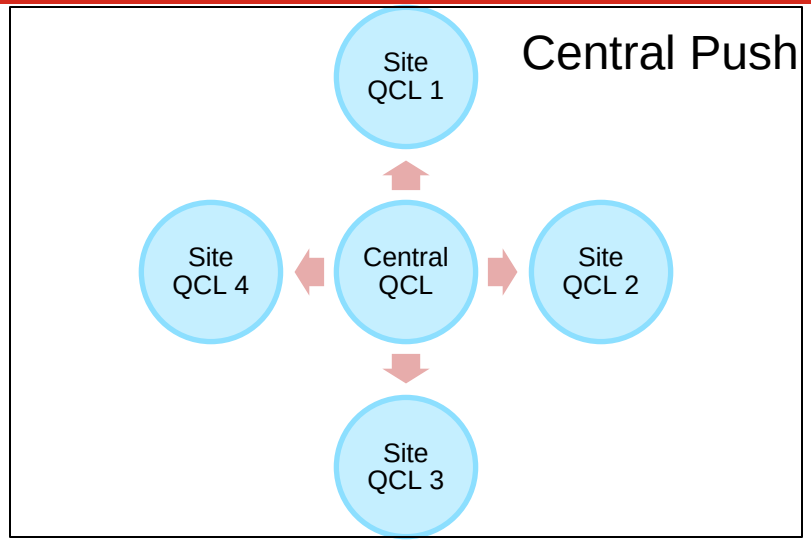
Guidance

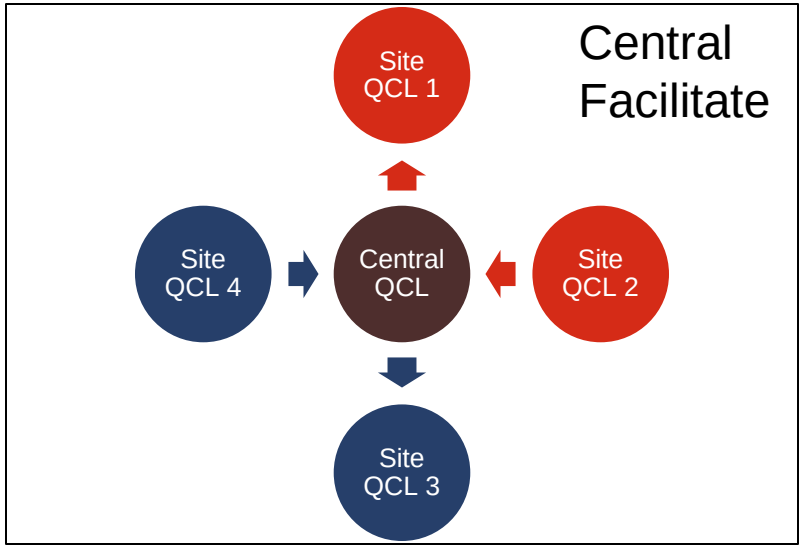
- Reducing Animal Impact
  - Internal Corporate Policy: Animal Use and Care Principles
  - European Union Directive 2010/63/EU
- Regulatory Guidance
  - 2012 FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers (Question 5)
  - Generally, compendia allow the use of appropriately validated non-compendia methods
    - USP <1225> Validation of Compendial Procedures (ICH)
    - Ph.Eur 5.1.10 Guidelines for using the test for bacterial endotoxins
    - Japanese Pharmacopoeia 16 General Notice 13
- Literature
  - Loverock, B. et. al. Stimuli to the Revision Process: A Recombinant Factor C Procedure for the Detection of Gram-negative Bacterial Endotoxin. 36(1), Jan-Feb. 2010

Lilly Status

- Method
  - The global method and associated infrastructure are active
  - 20 items are validated
    - 2 items (DP/DS) are transferred to QC labs
  - 13 items are ready to validate
  - 20+ items are in queue for method development
- Sanctioning
  - Alignment was gained at water and specification governance committees to implement the method
- Equipment
  - Platform equipment has been identified
  - Equipment is qualified at 3 sites; 2 more in 2016; 2-5 in 2017
- Compendia

Validation and Transfer Strategy Infrastructure





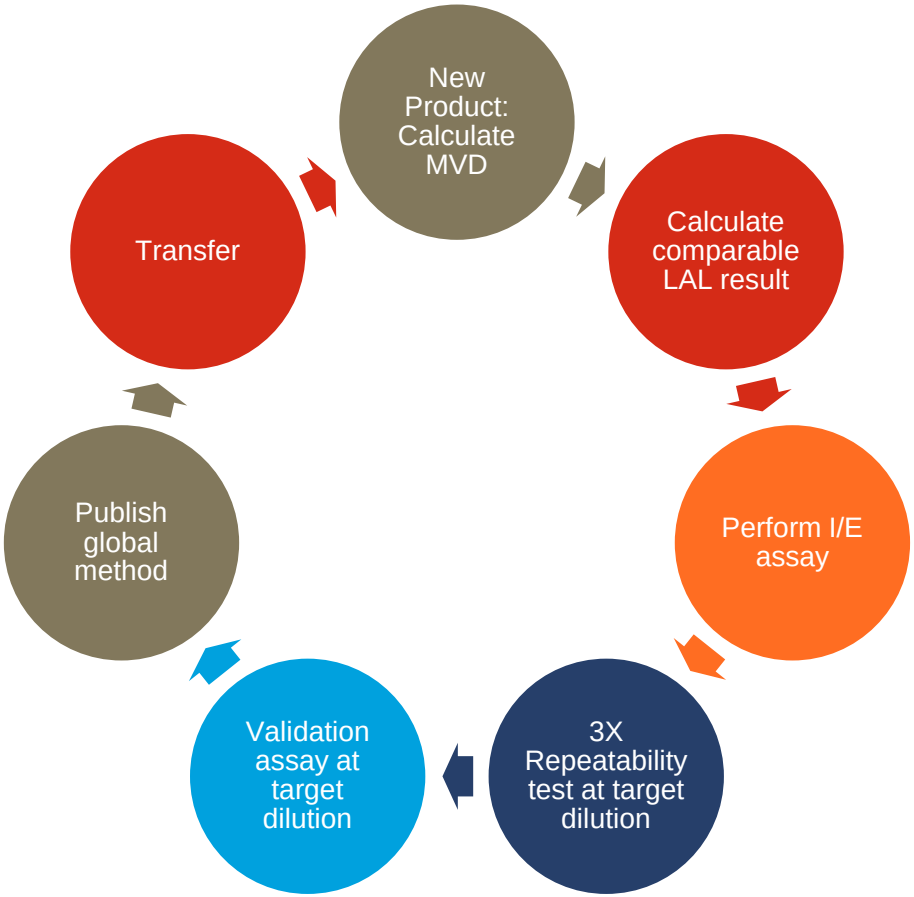
- Validation
  - Occurs in central QCL or at a site QCL
  - Site validations may be promoted to the global method using global standard curve, labware, document formats, etc.
  - Implementation controlled by multi-site change control
  - Multiple items can be grouped in a single change control for efficiency
    - items can be transferred “a la carte”
- Transfer
  - Central QCL transfers or facilitates transfer of centrally or site-generated method to other site QCLs
  - Simplified compared to compendia method verification
  - One central validation; transfer consists of repeating limited validation parameters, with or without training
  - 1 site invests more than typical; other sites less
- **Achieves common, global methods across the corporation**

Validation Strategy

Parameter	Source	Data
Accuracy	USP 1225	Lilly Validation
Precision	USP 1225	Lilly Validation
Inhibition/Enhancement	USP 85	Lilly Validation/Every Test
pH Suitability	USP 85	Lilly Validation
Linearity	USP 1225	Published Data/Every Test
Range	USP 1225	Published Data/Every Test
Specificity	USP 1225	Published Data/Every Test
LOQ	USP 1225	Published Data/Every Test
Robustness	USP 1225	One-time Lilly study

- A USP Stimuli to the Revision Process article was published demonstrating equivalency and/or superiority to the existing USP kinetic methods per USP 1225
- Linearity, Range, Specificity and LOQ are leveraged
  - Not product-specific
  - Data generated on each test

Validation Cycle



- I/E assay evaluates multiple dilutions across the MVD
- Result may or may not be equal to LAL result but must be within the product specificaiton

Robustness

Parameter	Type
Sensitivity Setting: nominal value, ± 3	Equipment
Sample/Reagent Volume: 100 µL ± 10% (100 µL of both sample and reagent ±10%)	Equipment
Incubation Time: 10 min., +10 min., +20 min.	Time
Time to Reagent Addition: 0 min., +10 and +20 min. at room temp., light-protected	Time

- Designed robustness based on extensive experience investigating common lab deviations
- Assay is robust at extremes of all studied parameters
- Improved Quality confidence

Parameter	Method Acceptance Criteria	Minimum Result From Study	Maximum Result From Study
Correlation coefficient	Within 0.980-1.000	0.999	1.000
Slope	Between 0.500 and 1.500	0.931	0.951
Y-intercept	Between 2.500 and 5.000	3.394	3.923
%CV of test replicates	NMT 25%	n/a	5%
0.5 EU/mL result	50-200%	110%	125%

Equipment & Software

- Lilly evaluated four different readers to date
  - Modified BioTek FLx800
  - Molecular Devices M5e
  - BioTek Synergy 2
  - Modified BioTek H1 (Pyrowave™)

- Existing Lonza WinKQCL™ software platform

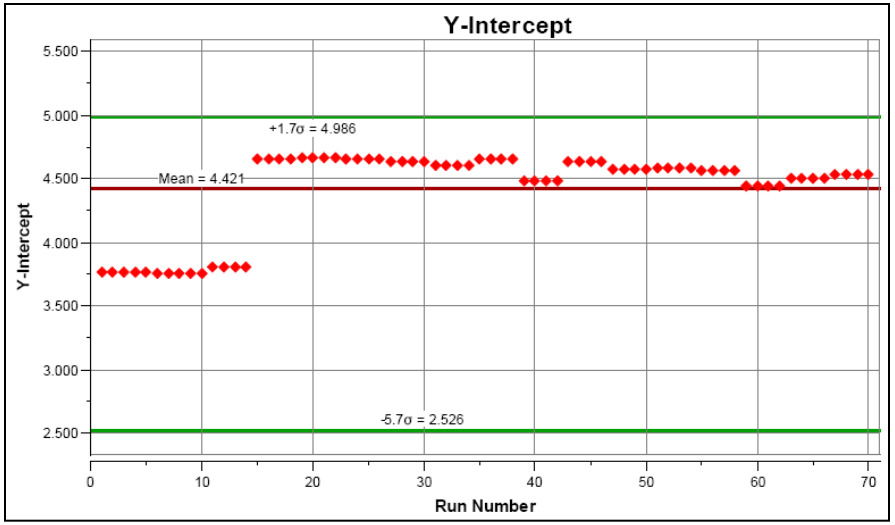
FLX800 / PyroWave Comparability

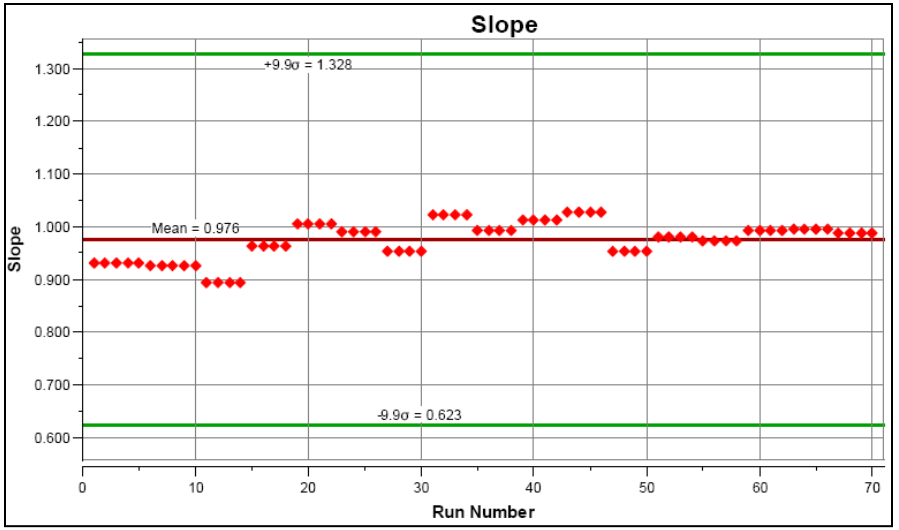
Item	Lot	FLX800 %PPC	Pyrowave %PPC
100 U/mL Insulin Solution	1	96	101
	2	101	90
	3	90	109
100 U/mL Insulin Mix Solution	1	86	83
	2	97	91
	3	96	75
100 U/mL Insulin Solution	1	90	115
	2	98	93
	3	101	113
100 U/mL Insulin Mix Suspension	1	100	81
	2	99	84
	3	95	78
100 U/mL Insulin Mix Suspension	1	93	107
	2	90	100
	3	90	96

Item	Lot #	FLX800 %PPC	Pyrowave %PPC
MAB, 3mg/mL	1	98	99
MAB, 10 mg/mL	1	106	104
	2	107	113
	3	106	110
MAB, 16 mg/mL	1	103	104
	2	107	101
	3	117	119
MAB, 80 mg/mL	1	102	93
	2	93	99
	3	105	95
MAB, 10 mg/mL	1	95	118
	2	102	103
	3	99	120

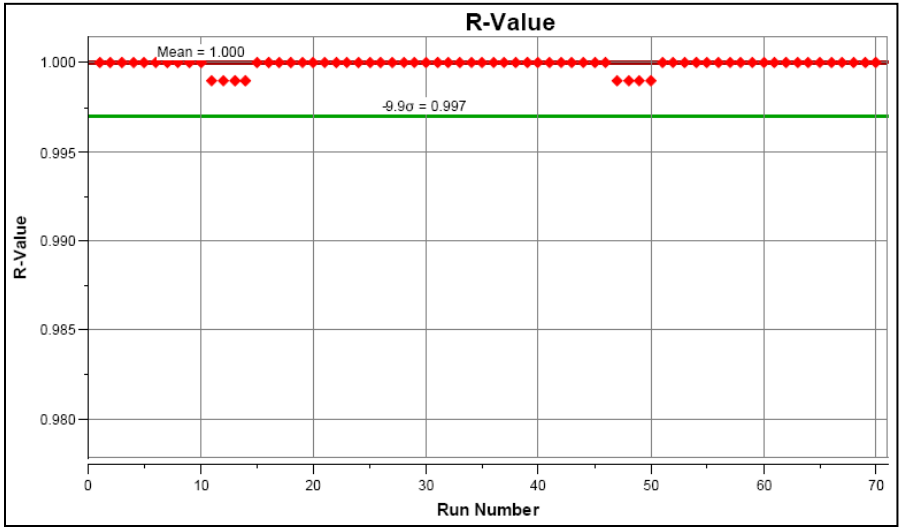
- PPCs are comparable between the FLX800 and PyroWave

rFC Performance: 0.01λ Standard Curve

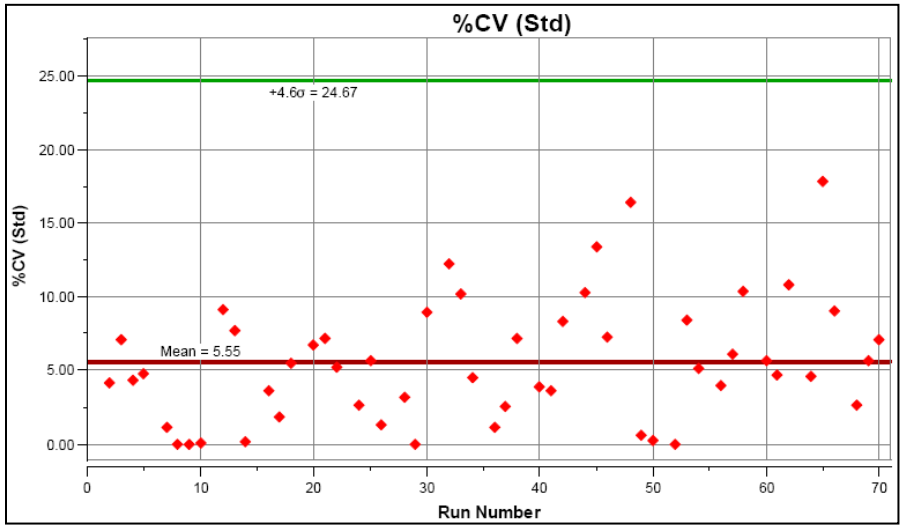


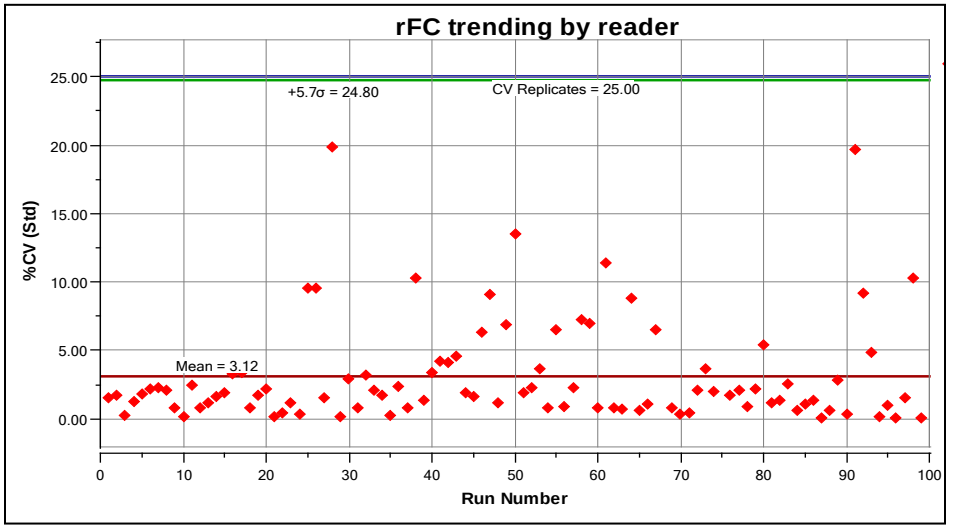


- Y-int, slope and linearity criteria are all operating well within vendor criteria (n>70 runs)



rFC Performance: 0.01λ Standard Curve %CV

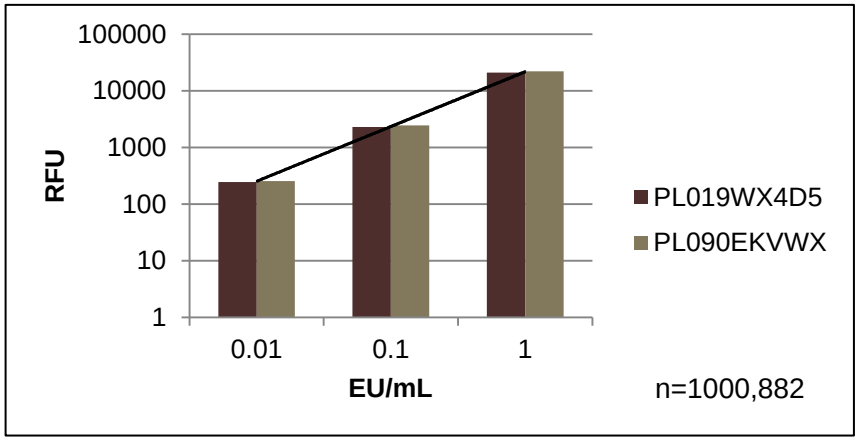




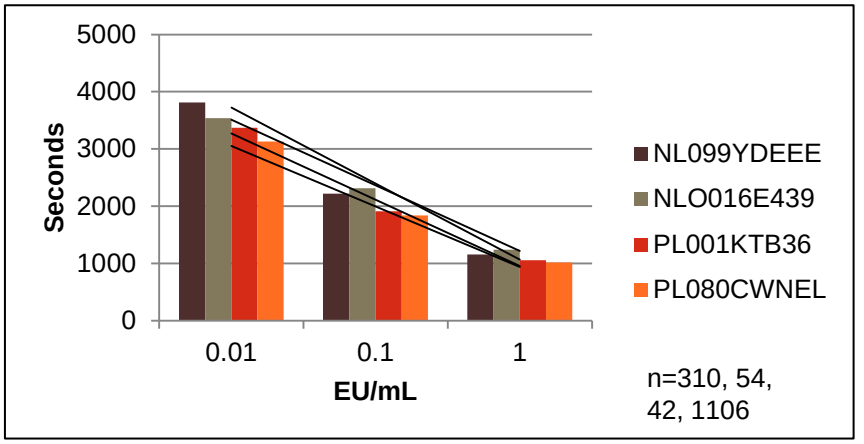
- Curve %CVs are within vendor <25% criteria
  - This is not assay precision and reflects an inherently more sensitive fluorescence assay at the cost of a noiser baseline
- Averaged 5.5% CV on initial platform instrument; 3.1% CV on the new platform instrument
- No more “hot wells”; all wells react
  - A slow/fast well will manifest itself as a %CV fail

rFC Standard Curve
Kit-to-Kit Variation

rFC Kits



LAL Kits



- 2 bulk rFC kits were used to date
  - The average standard curves between the 2 kits are comparable
- A recombinant protein manufactured by a validated process should yield more consistent curves compared to animal-sourced protein

Specificity: Case Study

Sample	Site1 LAL Result (EU/mL)	Site 2 LAL Result (EU/mL)	rFC (EU/mL)	GB Buffer +LAL (EU/mL)	PTS (pg/mL)
1	0.141	0.0946	<0.08	<0.04	2265
2	0.12	0.0712	<0.04	<0.04	1254
3	0.105	0.0748	<0.02	<0.04	970
4	0.132	0.157	<0.02	<0.08	3173
5	0.176	0.109	<0.04	<0.08	2798
LRW	Below detection limit				<10

- Low level endotoxin results reported at the end of a biotech drug substance process; confirmed at a second site
- rFC reported non-detect; confirmed by use of beta-glucan blocking buffer in LAL test, and orthogonal glucan detection test cartridge
- Factor C shown to not be susceptible to false positive factor G pathway

Water: Validation Results & Method Comparability

	LAL	rFC1	rFC2	
Batch 1 Result (EU/mL)	<0.05	<0.05	<0.05	Result Comparability
Batch 2 Result (EU/mL)	<0.05	<0.05	<0.05	
Batch 3 Result (EU/mL)	<0.05	<0.05	<0.05	
Batch 4 Result (EU/mL)	<0.05	<0.05	<0.05	
Batch 1 PPC (%)	148%	93%	88%	
Batch 2 PPC (%)	146%	114%	89%	Spike Comparability
Batch 3 PPC (%)	146%	112%	90%	
Batch 4 PPC (%)	154%	101%	95%	
Accuracy High (%)	compendia	100%	117%	
Accuracy Low (%)		116%	112%	Validation Results
Precision High (%)		4%	7%	
Precision Low (%)		12%	9%	

- Comparable results observed with and without analyte
- Two rFC vendor sources validated

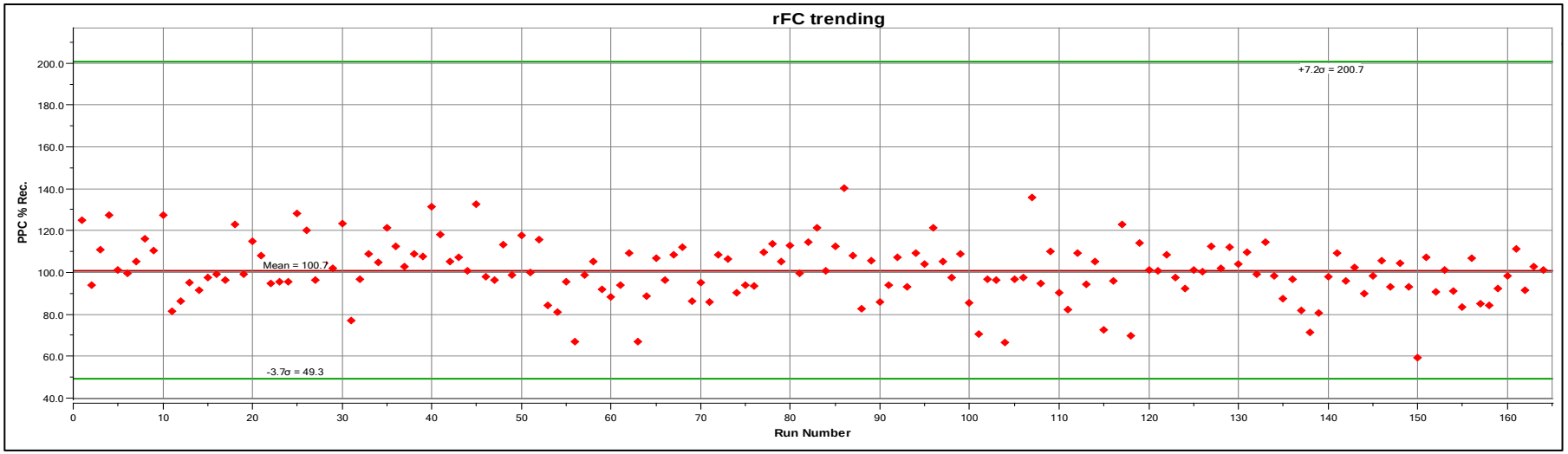
Water: 14 Day Hold Studies

Sample	LPS Recovery %				
	Day 0	Day 1	Day 4	Day 7	Day 14
Batch A; Polystyrene	100%	97%	100%	95%	97%
Batch B; Polystyrene	100%	97%	97%	97%	103%
Batch C; Polystyrene	100%	92%	96%	99%	99%
Batch A; PETG	100%	92%	92%	99%	115%
Batch B; PETG	100%	95%	105%	105%	109%
Batch C; PETG	100%	107%	103%	99%	107%

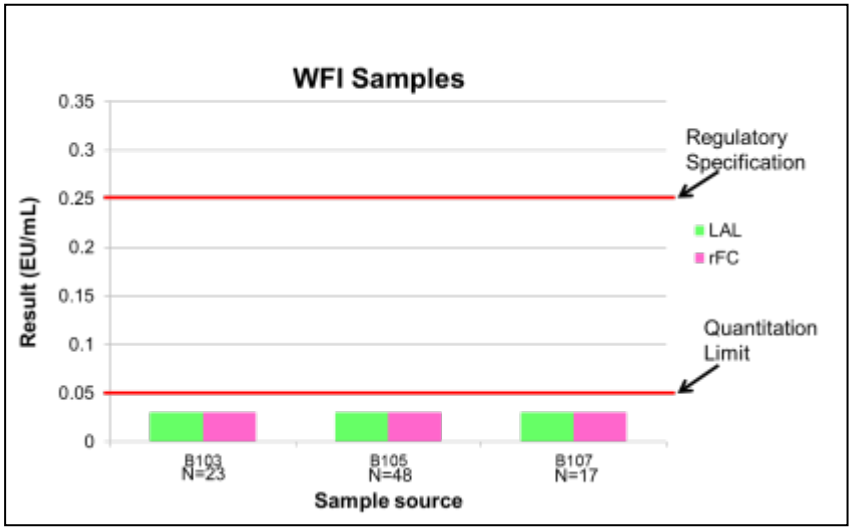
Sample	NOE Recovery %			
	Day 0	Day 4	Day 7	Day 14
Batch A; Polystyrene	100%	93%	89%	102%
Batch B; Polystyrene	100%	97%	93%	107%
Batch C; Polystyrene	100%	109%	95%	119%
Batch D; Polystyrene	100%	108%	103%	121%

- Water sample hold times were demonstrated to 14 days with both LPS and an E. coli O55:B5 NOE

Water: PPC & Endotoxin Activity



- PPC result mean (100.7%) is at target in over 160 samples
- rFC returns comparable results to LAL
- Mechanism of action is nearly identical; rFC does not detect endotoxin that LAL did not detect



Monoclonal Antibody rFC / LAL Comparability

	Result (all batches)	%PPC (avg.)	%CV (avg.)	N
Buffer Tank Endotoxin rFC (EU/mL)	<0.21	91%	13%	14
Buffer Tank Endotoxin BET (EU/mL)	<1.00	111%	13%	8
Pre-Filtration Drug Product Endotoxin rFC (EU/mg)	<0.00	84%	8%	14
Pre-Filtration Drug Product Endotoxin BET (EU/mg)	<0.02	98%	15%	8
Final Drug Product Endotoxin rFC (EU/mg, Placebo EU/mL)	<0.00	94%	7%	13
Final Drug Product Endotoxin BET (EU/mg, Placebo EU/mL)	<0.01	98%	20%	7

- Methods are comparable in a monoclonal antibody drug product buffer, in-process test and final release test with and without the presence of analyte

Insulin
rFC / LAL Comparability

	Insulin Solution		Insulin Suspension		Insulin Suspension	
	LAL	rFC	LAL	rFC	LAL	rFC
Batch 1 Result (EU/mg)	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U
Batch 2 Result (EU/mg)	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U
Batch 3 Result (EU/mg)	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U	<20 EU/100U
Batch 1 PPC (%)	114%	90%	113%	100%	97%	93%
Batch 2 PPC (%)	115%	98%	118%	99%	104%	90%
Batch 3 PPC (%)	102%	101%	99%	95%	113%	90%
Accuracy High (%)		92%		90%		87%
Accuracy Low (%)		89%		88%		90%
Precision High (%)		6%		9%		11%
Precision Low (%)		9%		10%		19%
pH (units)		historical		historical		historical

- Methods are comparable in insulin drug products with and without the presence of analyte

Monoclonal Antibody rFC / LAL Comparability

	Monoclonal Antibody DS		Monoclonal Antibody DP	
	LAL	rFC	LAL	rFC
Batch 1 Result (EU/mg)	<0.01	<0.01	<0.00883	<0.0042
Batch 2 Result (EU/mg)	<0.01	<0.01	<0.0100	<0.0042
Batch 3 Result (EU/mg)	<0.01	<0.01	<0.0200	<0.0042
Batch 1 PPC (%)	143%	99%	72%	104%
Batch 2 PPC (%)	134%	95%	84%	96%
Batch 3 PPC (%)	155%	98%	67%	102%
Accuracy High (%)		107%		102%
Accuracy Low (%)		98%		102%
Precision High (%)		4%		12%
Precision Low (%)		10%		13%
pH (units)		7.73-7.97 (rFC)		7.73-7.91 (rFC)

- Methods are comparable in monoclonal antibody drug product and drug substance with and without the presence of analyte

Monoclonal Antibody 2 rFC / LAL Comparability

	Monoclonal Antibody DS		Monoclonal Antibody DP	
	LAL	rFC	LAL	rFC
Batch 1 Result (EU/mg)	<0.01	<0.06	<0.02	<0.05
Batch 2 Result (EU/mg)	<0.01	<0.05	<0.02	<0.05
Batch 3 Result (EU/mg)	<0.01	<0.06	<0.02	<0.05
Batch 1 PPC (%)	99%	93%	100%	80%
Batch 2 PPC (%)	81%	96%	105%	93%
Batch 3 PPC (%)	71%	99%	73%	90%
Accuracy High (%)		90%		86%
Accuracy Low (%)		77%		80%
Precision High (%)		4%		4%
Precision Low (%)		5%		6%
pH (units)		7.77-7.90 (rFC)		7.80-7.97 (rFC)

- Methods are comparable in monoclonal antibody drug product and drug substance with and without the presence of analyte

Raw Materials
rFC / LAL Comparability

	Dibasic Sodium Phosphate		Glycerin Synthetic	
	LAL	rFC	LAL	rFC
Batch 1 Result (EU/mg)	0.0423	<0.01	<0.005	<0.01
Batch 2 Result (EU/mg)	<0.0025	<0.01	<0.005	<0.01
Batch 3 Result (EU/mg)	<0.0025	<0.01	<0.005	<0.01
Batch 1 PPC (%)	55%	114%	116%	90%
Batch 2 PPC (%)	120%	117%	103%	95%
Batch 3 PPC (%)	124%	119%	108%	91%
Accuracy High (%)		111%		94%
Accuracy Low (%)		93%		90%
Precision High (%)		4%		8%
Precision Low (%)		3%		6%
pH (units)		7.31-7.37 (dilution)		6.78-7.05 (dilution)

- Methods are comparable in two raw materials with and without the presence of analyte

Conclusion

- For Lilly, moving to the end-point fluorescence technique using rFC is advantageous
- The end-point fluorescence technique using rFC is robust, accurate, precise and has been shown to be equivalent or superior to the compendia method
- The end-point fluorescence technique using rFC should be incorporated into the compendia method chapters