



Endosafe[®] Trillium[®] Recombinant Cascade Reagent Validation Approach

Alan Hoffmeister

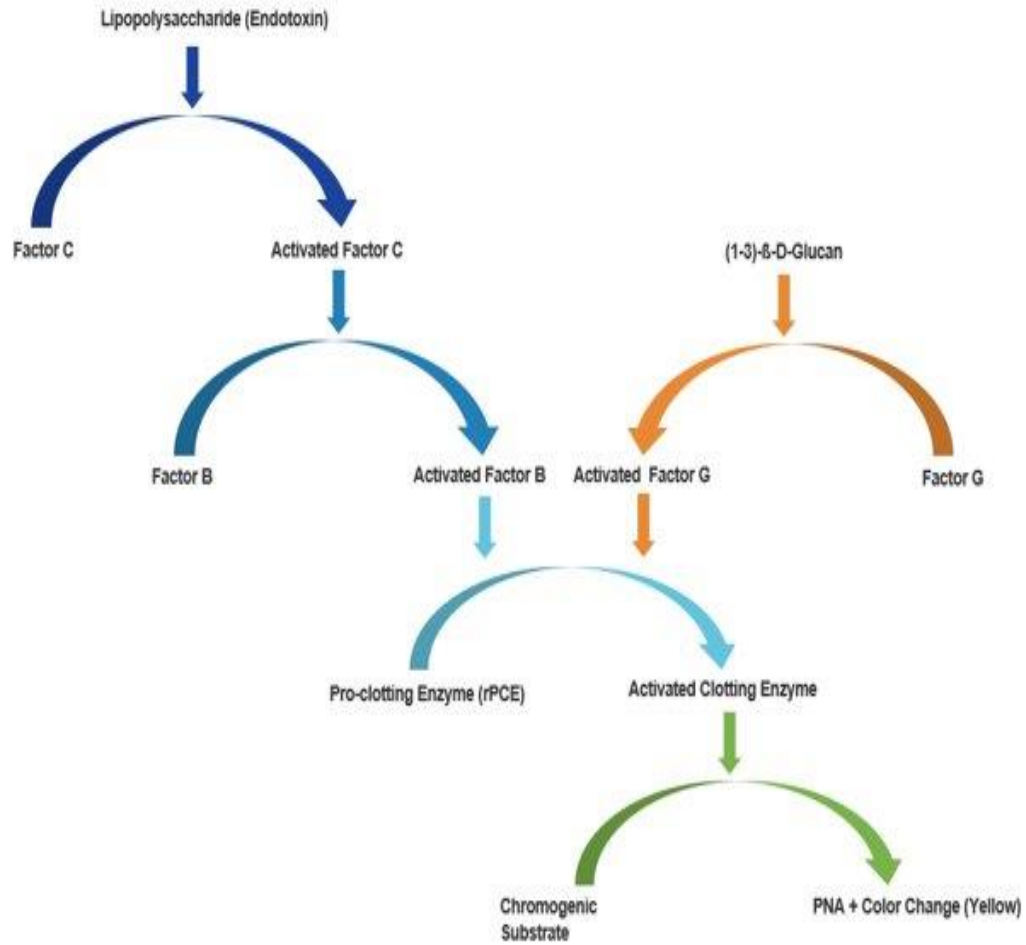
Senior Global Scientific Portfolio Specialist



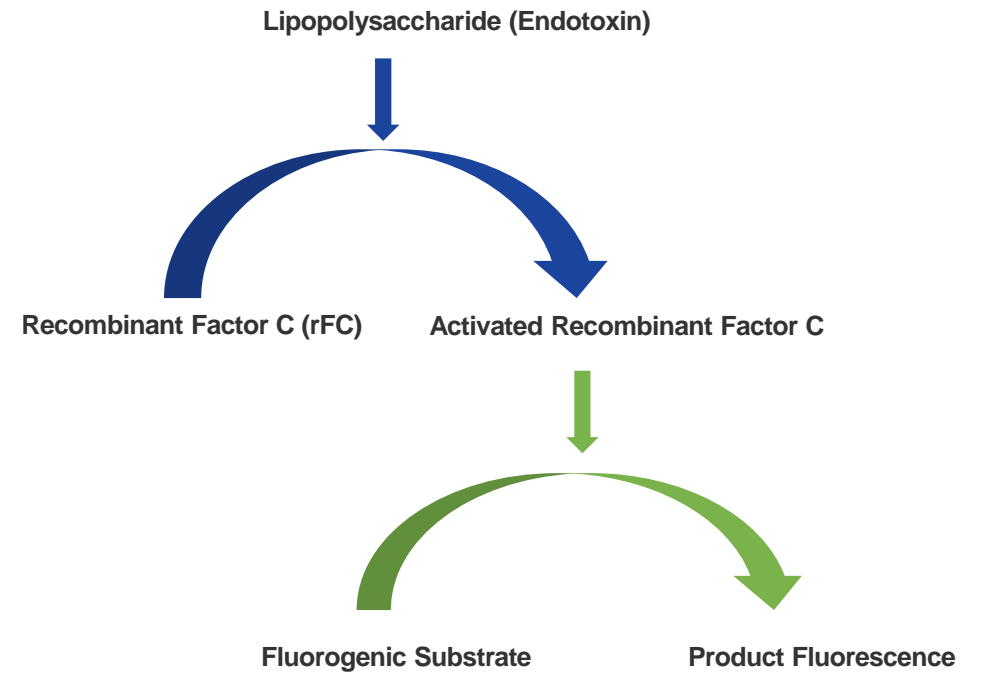
**Recombinant Factor C (rFC)
versus
Recombinant Cascade Reagent
(rCR)**



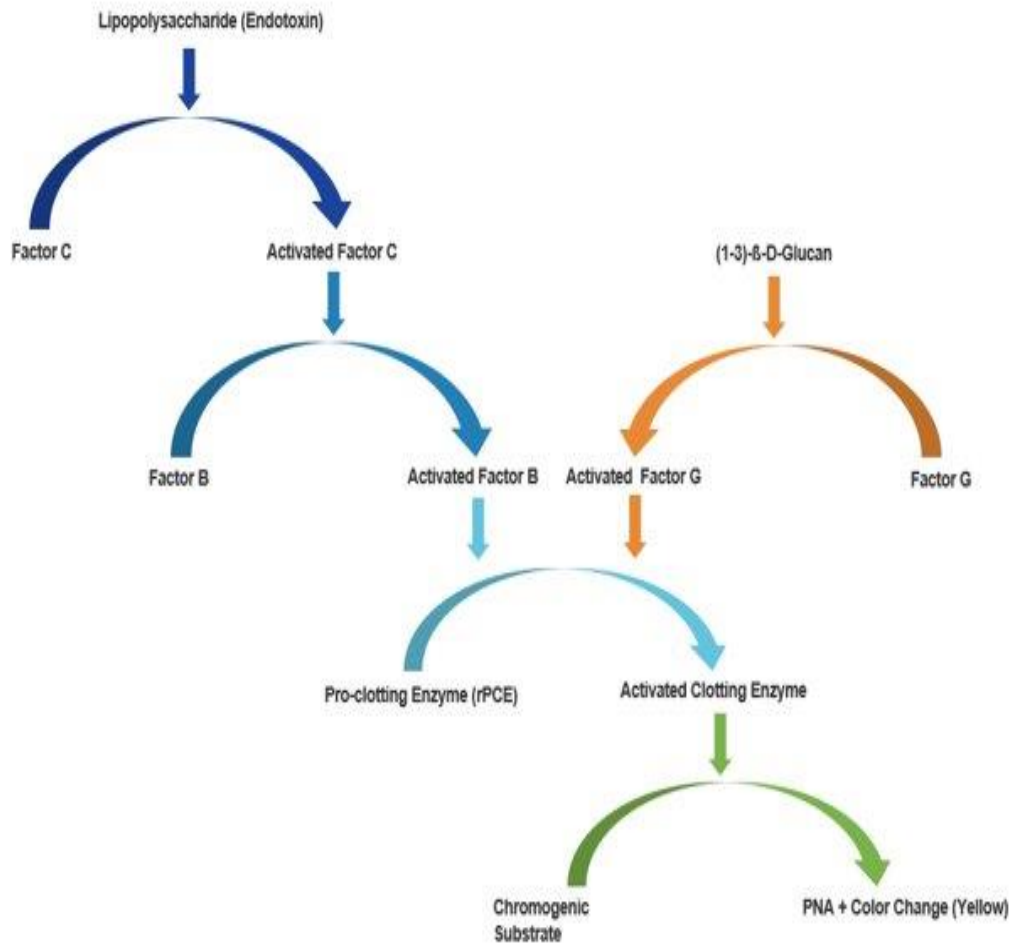
Natural LAL Cascade



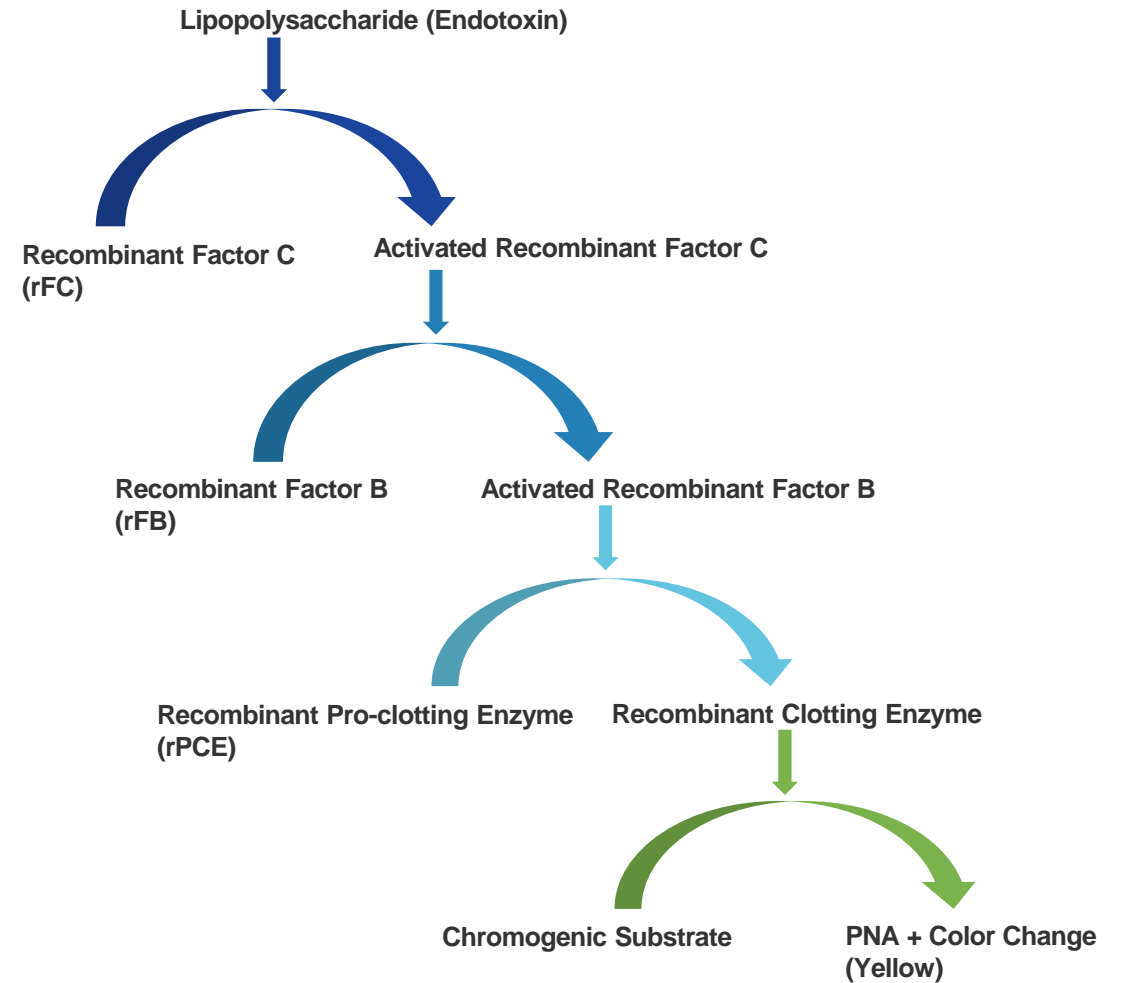
Recombinant Factor C



Natural LAL Cascade



Recombinant Cascade Reagent




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**Endosafe[®] Trillium[®]
Recombinant Cascade Reagent**



Advancing **Responsible** Science through Sustainable Endotoxin Testing Solutions

Endosafe LAL Cartridge Technology



- + **95% Reduction** in horseshoe crab blood from Gel-Clot.
- + FDA-Licensed and compliant with harmonized Pharmacopeia chapters.
- + Robust and scalable technology, from single test to full automation.

Endosafe Trillium Recombinant Cascade Reagent (rCR) Vial



- + **100% Replacement** in horseshoe crab blood.
- + Optimized kinetic chromogenic reagent formulated to simulate the natural LAL reaction.
- + Compatible with existing plate readers and supported by Endoscan-V software.

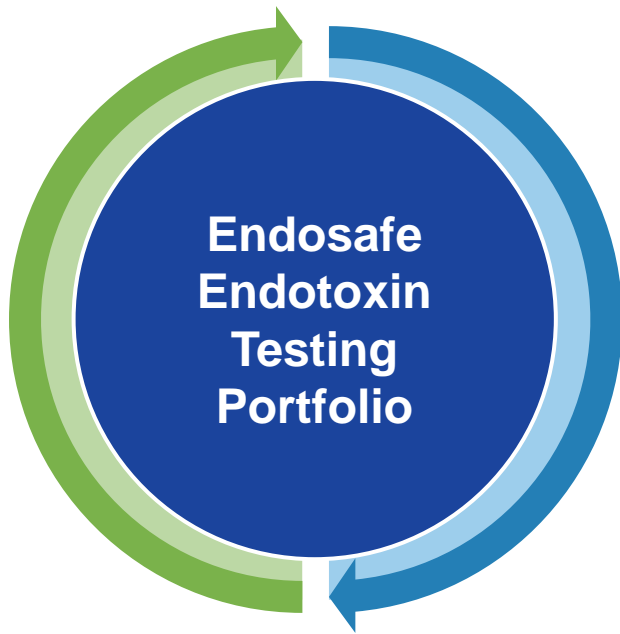
Endosafe Trillium rCR Cartridge Technology



- + **100% Replacement** in horseshoe crab blood.
- + Optimized kinetic chromogenic reagent formulated to simulate the natural LAL reaction.
- + Seamless integration with existing suite of scalable, automated, Endosafe cartridge technology platforms & Endoscan-V software.

Sustainable Endotoxin Testing Complete Solution

A complete, sustainable, and scalable BET Portfolio



Traditional
kinetic
chromogenic

KCA in vials
Reduction **54%¹** LAL

Trillium rCR vials
100% LAL Replacement



Cartridge
Technology
(advanced
Kinetic)

LAL cartridges
Reduction **95%¹** LAL

Trillium rCR cartridges
100% LAL Replacement



Conclusion for Interference Patterns

107 non-water samples tested

- 104 samples worked at the same dilution for both LAL / rCR
- 2 samples worked at a lower dilution for rCR (1:1 vs 1:10 & 1:1k vs 1:2k)
- 1 sample required further dilution for rCR (1:1k vs. 1:5k)*

rCR has similar interference patterns to LAL for > 99% of the samples tested.

* MVD unknown due to lack of completed information from manufacturer

<https://www.criver.com/resources/download-ms-endosafe-trillium-rcr-beta-study-technical-report>

Sample Category	Number Tested
Small Molecule	23
Raw materials	17
Plasmid	14
Lentivirus	11
Capsid	10
Injectables	9
Large Molecule	6
Biologicals	5
Vaccine	2
MAB	2
Adenovirus	1
Devices	1
Cell Supernatant	1
Other	3


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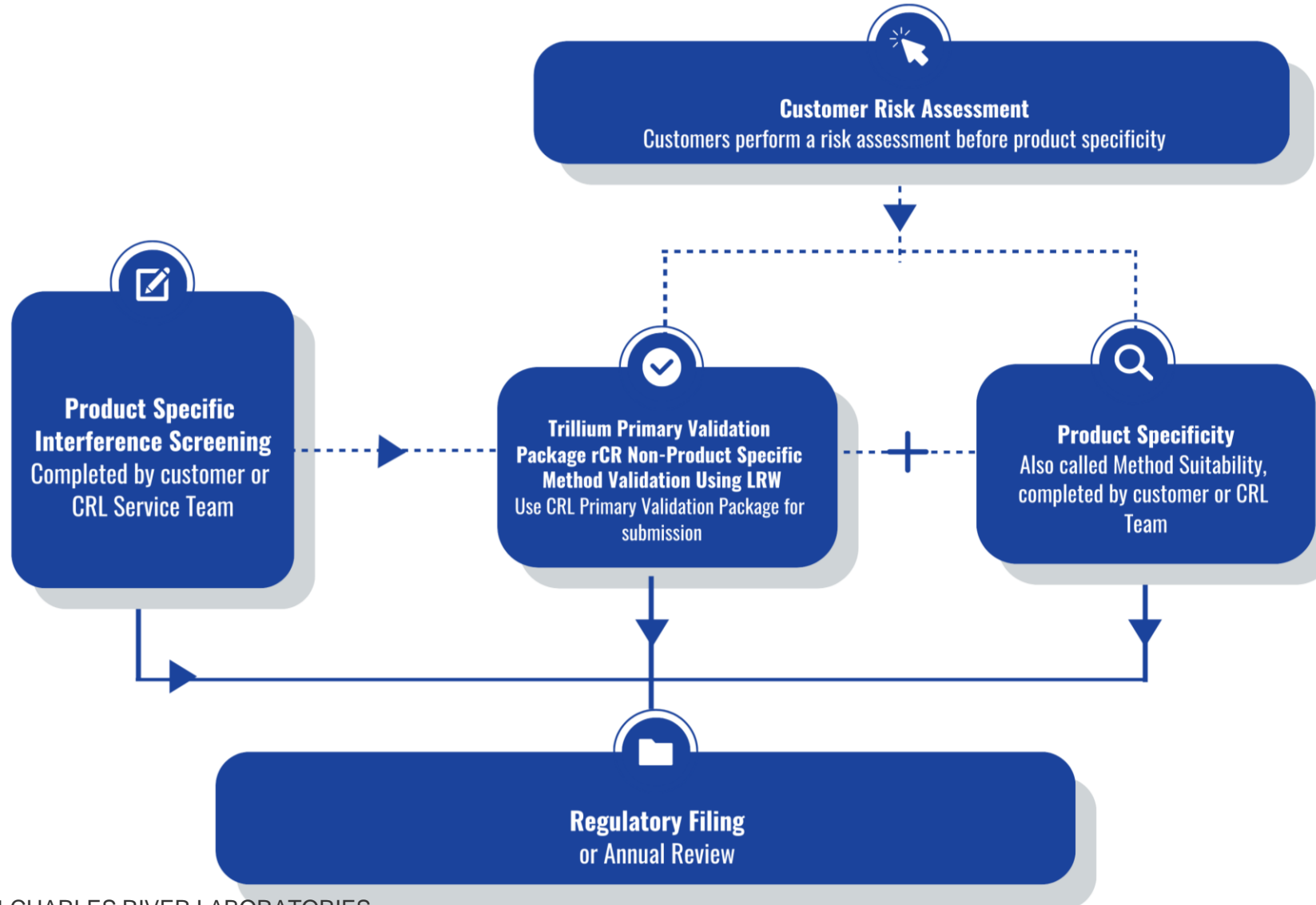
Endosafe[®] Trillium[®] Recombinant Cascade Reagent

Vials & Cartridges Validation Approach



Endosafe® Trillium – Validation strategy & Support Services

Vials & Cartridges



Endosafe® Trillium – Risk Assessment Potential Scenarios For Validation Strategy

Vials & Cartridges

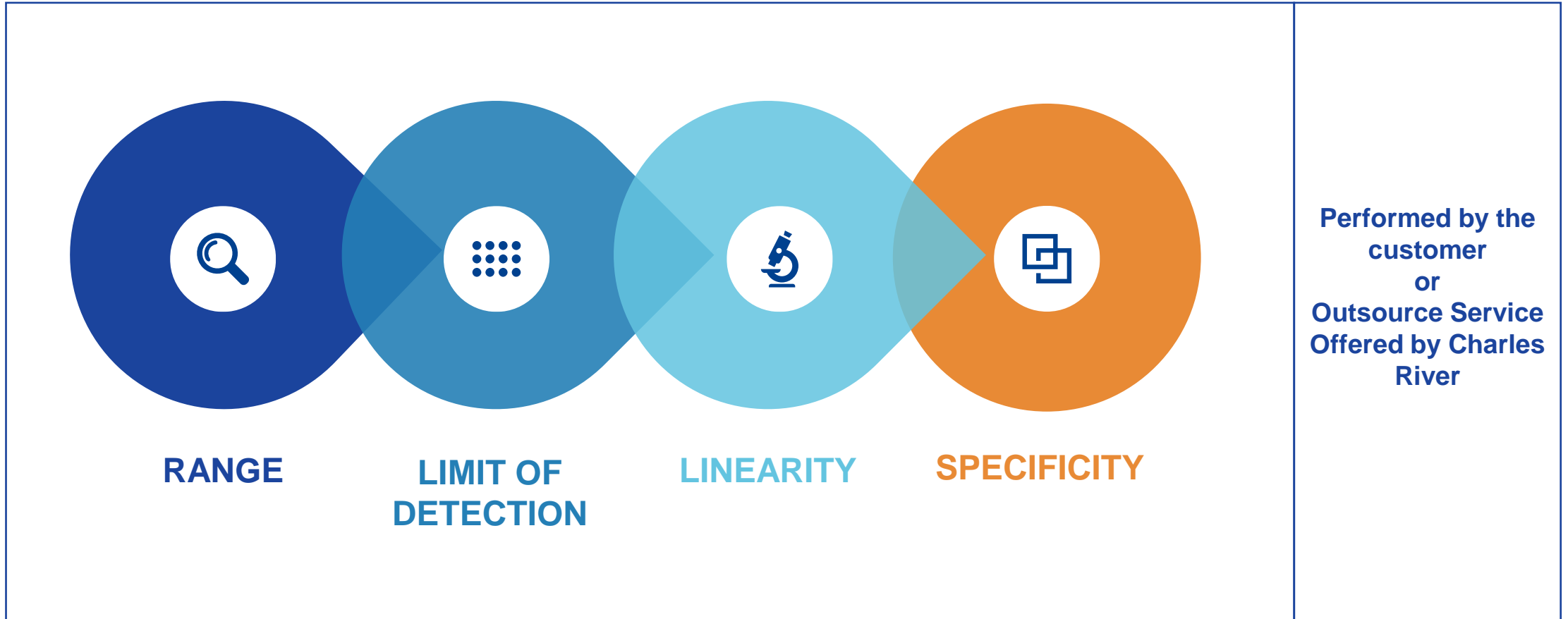
		Parameters required by ICH Q2 for an analytical method				
		Water		Product or intermediate (Dossier submitted)		
		WFI	PW	Approach 1	Approach 2	Approach 3
Accuracy						
Precision	Repeatability					
	Intermediate Precision					
Range						
Linearity						
Limit of detection						
Specificity						

Robustness	rCR only	rCR only	rCR only	rCR only	rCR only
Equivalency (rCR to LAL)					

	Trillium Primary Validation Package	Performed by Charles River with LAL and rCR
	Product Specific Validation	Performed by the customer with rCR

Product Specificity (Method Suitability)

CR recommended Parameters to be assessed on per product basis



CR strongly recommend the user perform a risk assessment to determine approach to validation

Product Specificity (Method Suitability)

CR recommended Parameters to be assessed on per product basis - Plate template example

Plate 1 EXAMPLE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SC 5EU/mL	SC 5EU/mL		SMP1	SMP1		SMP2	SMP2		SMP3	SMP3	
B	SC 0.5EU/mL	SC 0.5EU/mL		SMP 1.1 1EU/mL	SMP 1.1 1EU/mL		SMP 2.1 1EU/mL	SMP 2.1 1EU/mL		SMP 3.1 1EU/mL	SMP 3.1 1EU/mL	
C	SC 0.05EU/mL	SC 0.05EU/mL										
D				SMP1	SMP1		SMP2	SMP2		SMP3	SMP3	
E				SMP 1.2 0.5EU/mL	SMP 1.2 0.5EU/mL		SMP 2.2 0.5EU/mL	SMP 2.2 0.5EU/mL		SMP 3.2 0.5EU/mL	SMP 3.2 0.5EU/mL	
F	Neg Ctrl	Neg Ctrl										
G				SMP1	SMP1		SMP2	SMP2		SMP3	SMP3	
H				SMP 1.3 0.25EU/mL	SMP 1.3 0.25EU/mL		SMP 2.3 0.25EU/mL	SMP 2.3 0.25EU/mL		SMP 3.3 0.25EU/mL	SMP 3.3 0.25EU/mL	

Range & Linearity

Limit of Detection

Specificity

Performed by the customer or Outsource Service Offered by Charles River

Product Specificity (Method Suitability)

CR recommended Parameters to be assessed on per product basis - Example

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Certificate of Analysis

Range

Linearity

Reorder Code: RCRC3001 Cartridge Lot #: 2326213117 Expiration Date: Mar 2024
 Calibration Code: 119845654563018 RSE Lot #: H0K354

Archived Standard Curve Range: 1-0.01 EU/mL Standard Curve Linearity: -0.999

Standard Curve Mean Reaction Times: 1.0 EU/mL 198 seconds
LOD 0.1 EU/mL 456 seconds
0.01 EU/mL 946 seconds

Archived Spike Concentration: 0.100 EU/mL Negative Control: PASS

**NOT TO BE USED FOR ANY FORMAL, PHARMACEUTICAL RELEASE TESTING
 NOT FOR RESALE, EXPERIMENTAL USE ONLY**

This lot of PTS Cartridges has been tested and meets Quality Control testing requirements for an archived curve, negative controls, and positive product control results.

Store cartridges at 2-25°C. Allow the unopened foil pouch to reach room temperature (20-25°C) for a minimum of 8 hours prior to opening. Cartridges should be used immediately once the foil pouch seal has been opened. Cartridges are for single-test use only.

CAUTION: DO NOT FREEZE THE CARTRIDGES

Qualified Analyst: M. Slay Date: 22 Sep 2023
 Reviewed By: B. Chs Date: 22 Sep 2023

Charles River Laboratories, Inc.
 1023 Wappoo Road, Suite 43-B
 Charleston, SC 29407 USA

Specificity		
Sample batch 1	Sample batch 2	Sample batch 3
 <div style="background-color: #add8e6; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,2EU/mL </div>	 <div style="background-color: #add8e6; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,2EU/mL </div>	 <div style="background-color: #add8e6; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,2EU/mL </div>
 <div style="background-color: #0072bc; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,1EU/mL </div>	 <div style="background-color: #0072bc; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,1EU/mL </div>	 <div style="background-color: #0072bc; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,1EU/mL </div>
 <div style="background-color: #004a87; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,04EU/mL </div>	 <div style="background-color: #004a87; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,04EU/mL </div>	 <div style="background-color: #004a87; padding: 10px; border: 1px solid #0056b3;"> Sample diluted at working dilution and spiked at 0,04EU/mL </div>

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Regulatory Landscape for Recombinant BET



Global Regulatory Agencies Not Aligned

Current Position

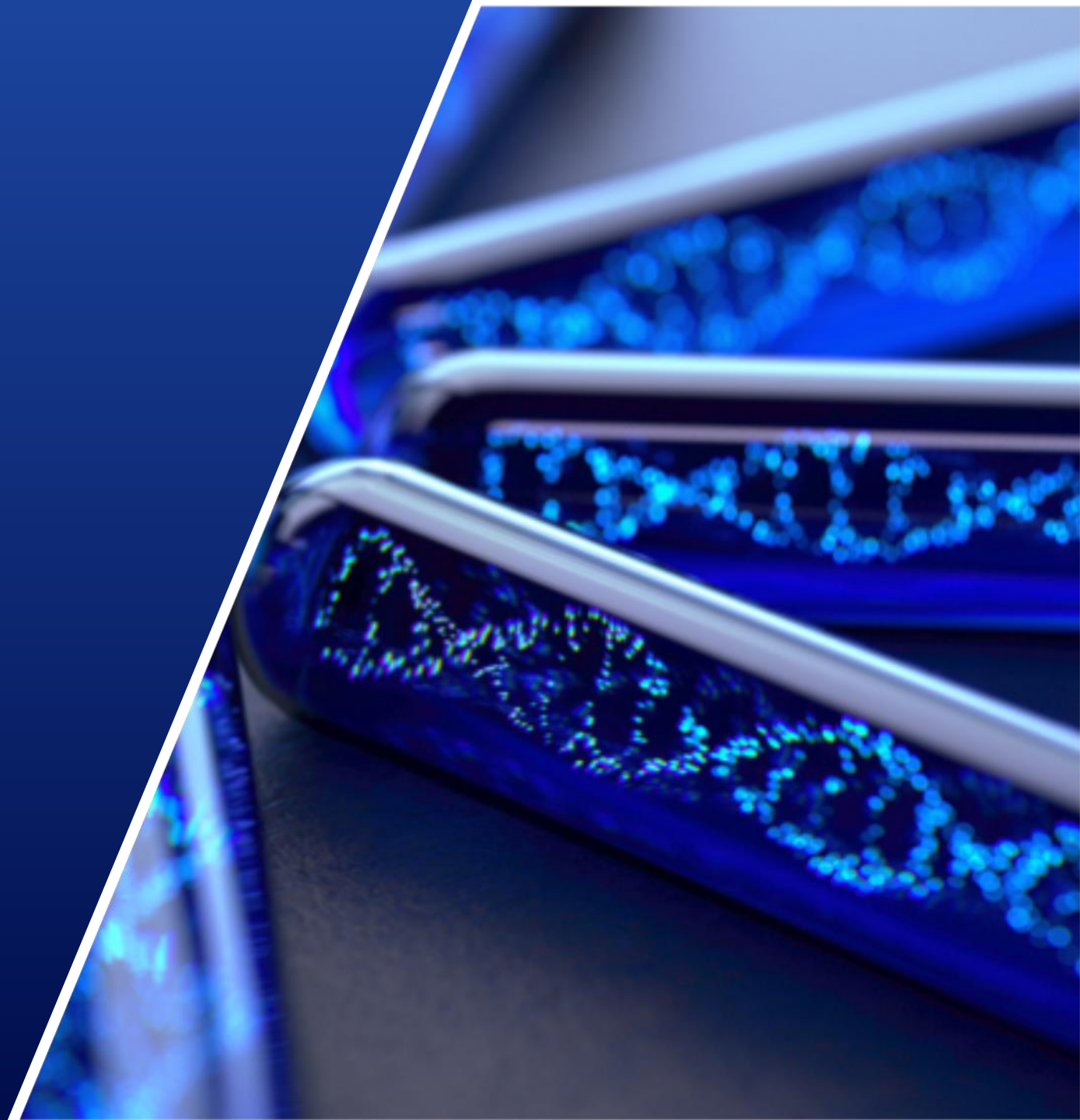
	Endotoxin Testing		
	LAL	rFC	rCR
Ph. Eur.	Accepted	Accepted*	In Discussion
WHO	Accepted	Proposed*	Alternative
USP	Accepted	Accepted*†	Accepted*†
Japan	Accepted	Alternative	Alternative
India	Accepted	Proposed*	Proposed*
China	Accepted	Alternative	Alternative
S. Korea	Accepted	Alternative	Alternative
Brazil	Accepted	Proposed*	Alternative

*Alternative method validation required, when the chapter is not referenced in mandatory product specific monographs.

†Chapter <86> approved 26 July 2024. Final text to be published November 2024 for early adoption and becomes official May 2025

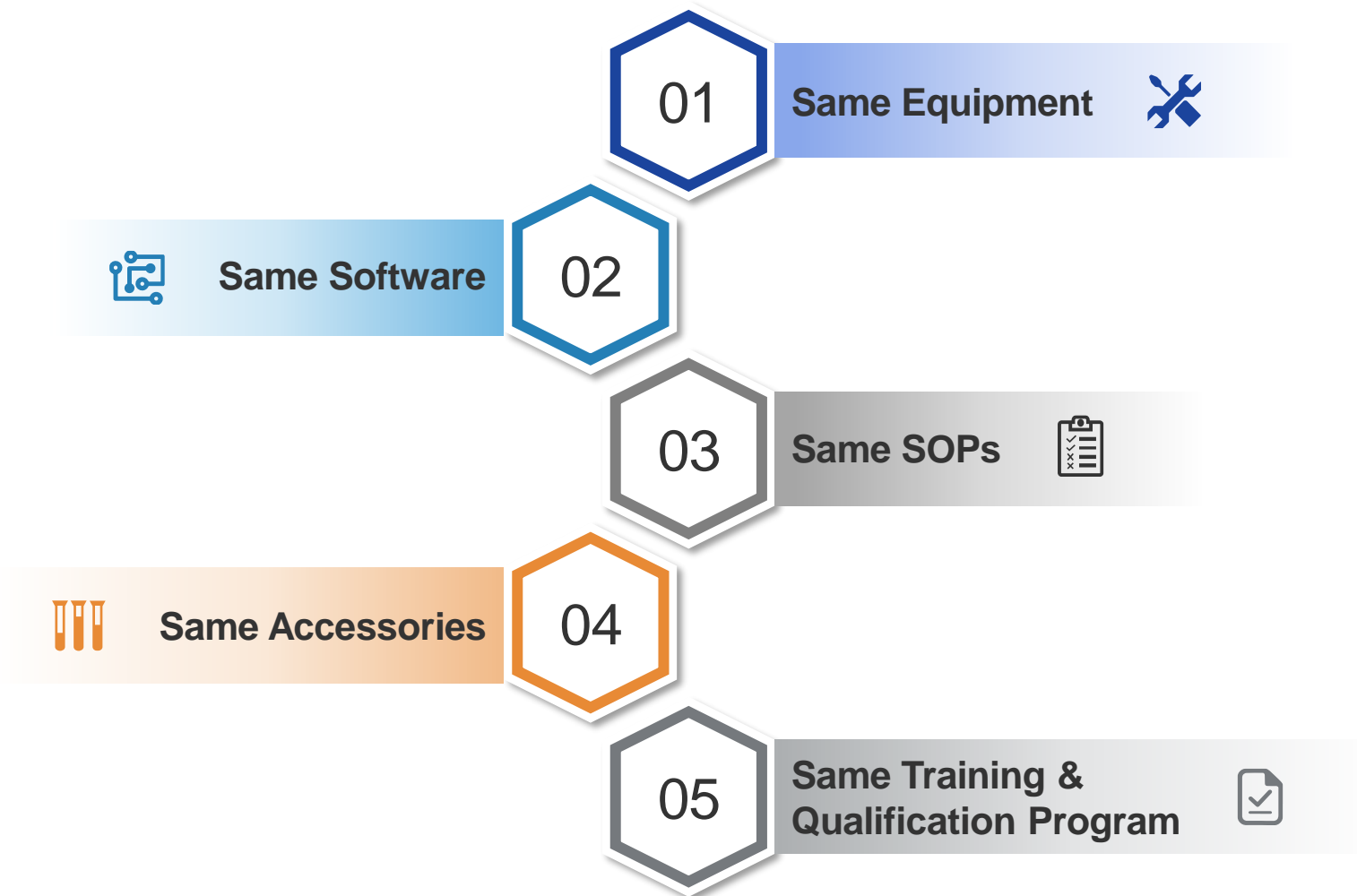

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Conclusions



Endosafe Trillium

The advantages of a recombinant cascade reagent (rCR)





Contact Us

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