

Microbial Contamination and Control Conference





Advancing Sustainability: Harnessing Recombinant Reagents for Endotoxin Detection

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Microbial Contamination and Control Conference

May 8th & 9th



Agenda

- Introduction
- Introduction to Bacterial Endotoxin
- BMS Commitment to Sustainability
- Recombinant Evaluation Approach
 - Points to Consider
- Review of Data
- Conclusion



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Introduction

Former Animal Educator and Emergency Service Technician

Bachelors in Biological Sciences

5 Years of Pharmaceutical Microbiology Experience

PDA poster presenter at 2023 PDA Micro conference and volunteer at the PDA Metro chapter

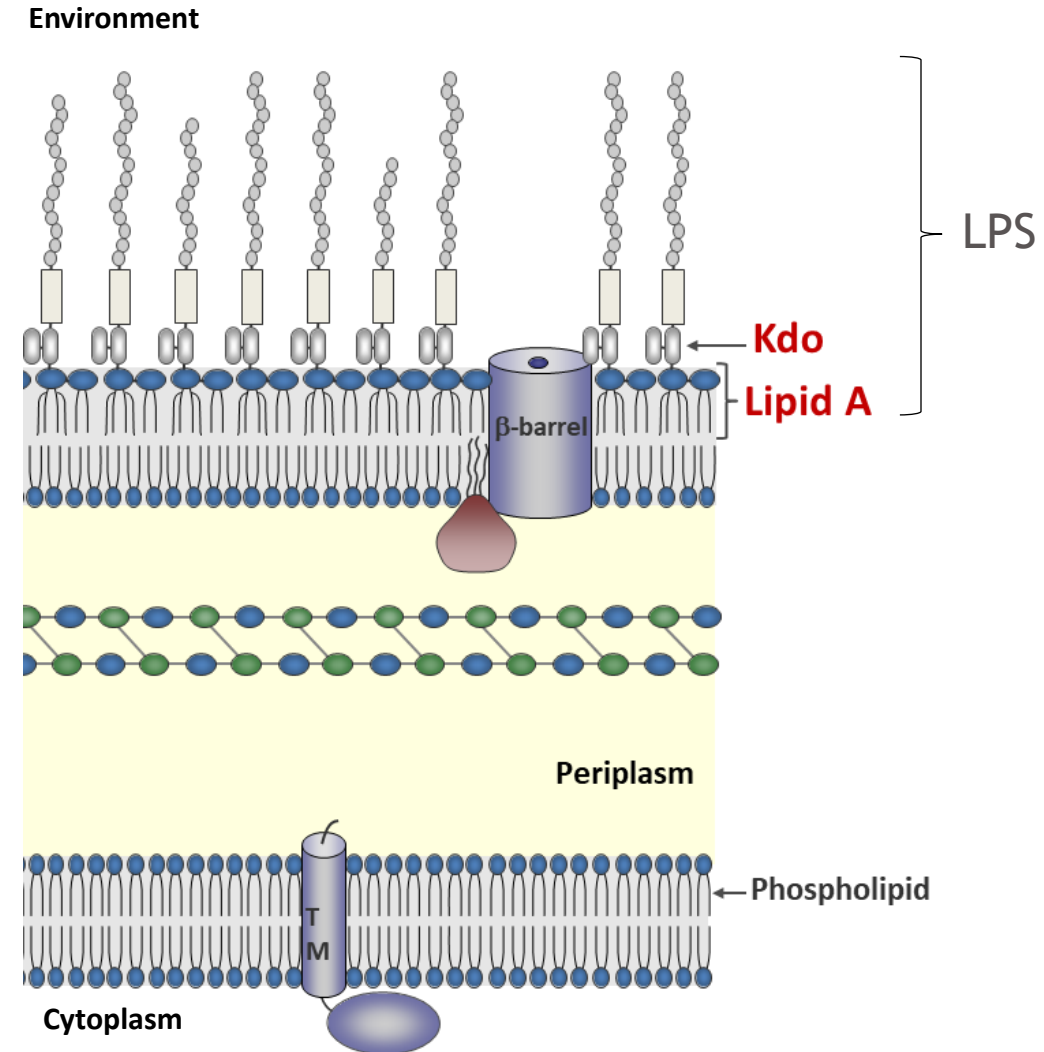
Endotoxin Lead for BMS Micro Center of Excellence performing 100s of assays for Low Endotoxin Recovery





Introduction to Bacterial Endotoxin

- Endotoxin may be referred to as lipopolysaccharide (LPS)
- LPS is the major component of the outer membrane of Gram-negative bacteria
- Endotoxin/LPS is responsible for pyrogenic responses in vivo
- May be associated with living cells or fragments of dead cells



Adapted from Trent, M. S. Microbial Glycobiology. ASM Press, 2008



Endotoxin and Regulations

- 21 CFR 211.167(a) states “For each batch of drug product purporting to be sterile and pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements.....”
- 21 CFR 610.13(b) references testing for pyrogenic substances, which states “Each lot of final containers of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits”



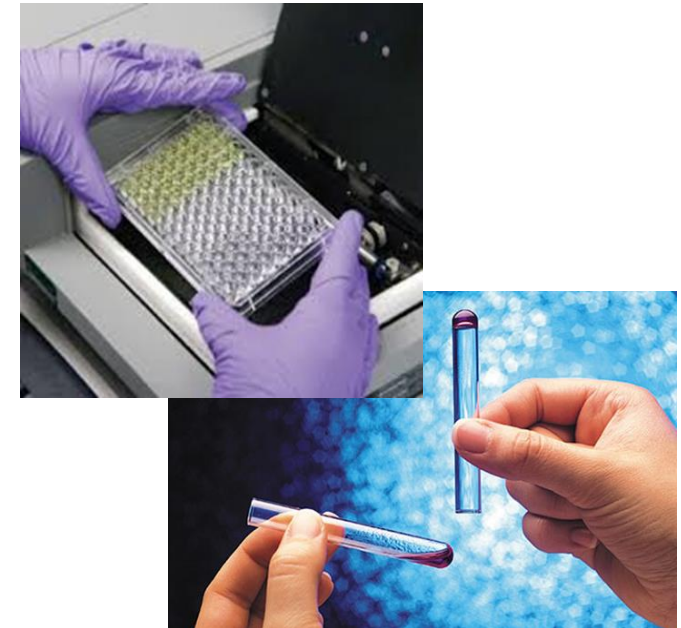
Bacterial Endotoxin Testing (BET)

- Testing is typically based on the clotting cascade of the horseshoe crab (*Limulus polyphemus*)
 - BET vendors may use the blood from horseshoe crabs to manufacture *Limulus* Amoebocyte Lysate (LAL) for endotoxin detection
- The LAL assay is the harmonized compendial test for endotoxin detection
 - USP 85
 - Ph. Eur. 2.6.14
 - JP 4.01



Types of BET

- 3 types of harmonized BET methods:
 - Chromogenic – yellow color
 - Turbidimetric – turbidity
 - Gel clot – clot formation
- Additional endotoxin testing:
 - Recombinant Factor C* – fluorescence end-point
 - Ph. Eur. 2.6.32
 - Recombinant Cascade Reagent* – chromogenic
 - Monocyte Activation Test – quantifies the cytokine concentration
 - Ph. Eur. 2.6.30

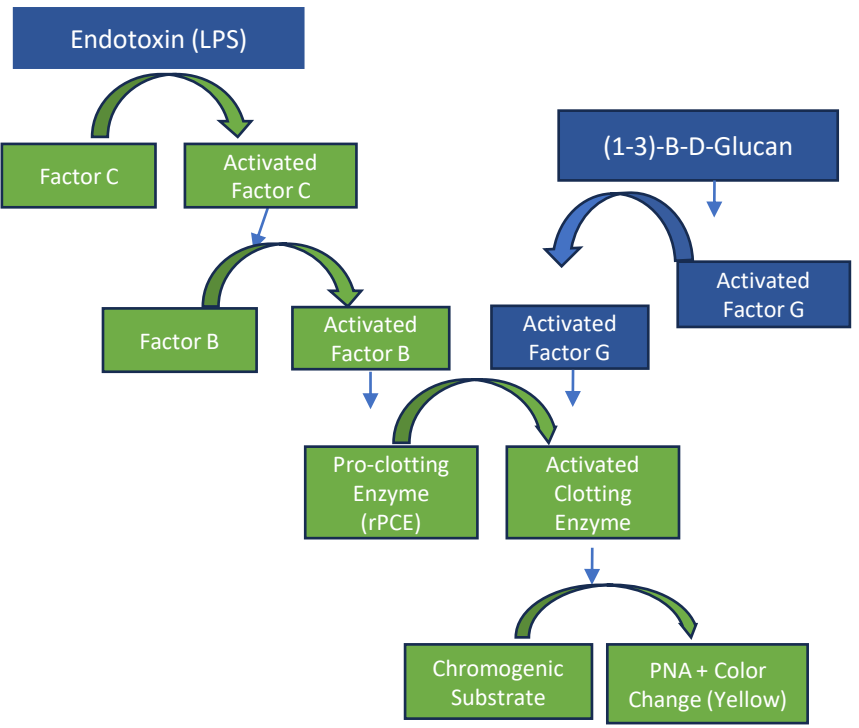


*USP <86> Bacterial Endotoxins Test Using Recombinant Reagents was published in PF 49(6)

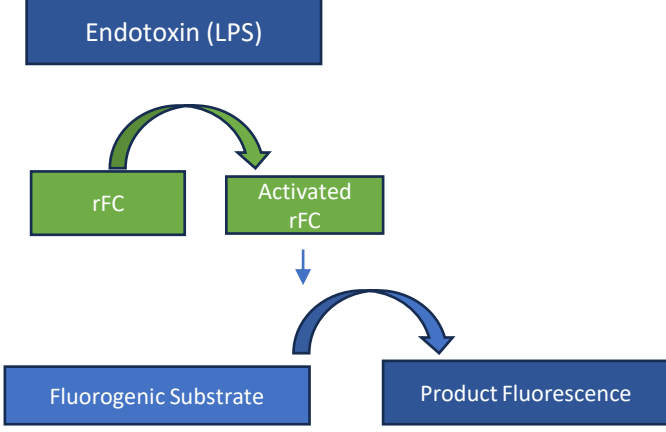


Cascade Comparison

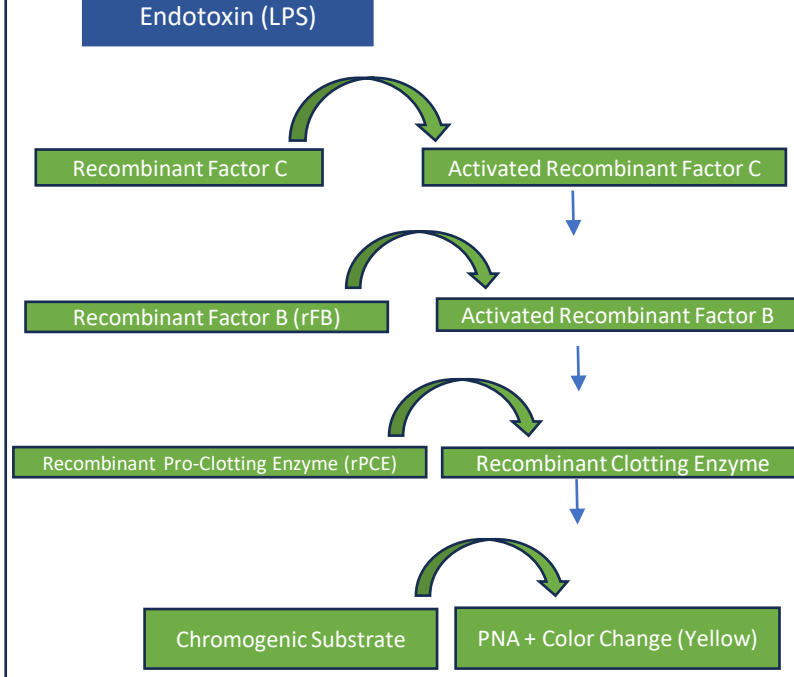
LAL Cascade



Recombinant Factor C (rFC)



Recombinant Cascade reagent (rCR)



Equivalent
Different



BMS Commitment to Sustainability

Building a Better Future:
Bristol Myers Squibb
2023 ESG Report

Synthetic Alternatives to Animal-Derived Reagents

Our mission to develop and deliver innovative medicines comes with an obligation to ensure they are safe and efficacious. As part of that obligation, BMS employs industry-standard microbial testing on injectable medicines prior to making them available to patients. One set of tests uses limulus amoebocyte lysate (LAL) reagents, derived from horseshoe crab blood, to detect endotoxins. Endotoxins are released by bacteria and have toxic effects on the human body, which can lead to complications that range from nausea, vomiting and diarrhea to intravascular coagulation and organ failure.

BMS takes seriously our responsibility for the ethical treatment of animals required for use in research and manufacturing, employing the 3R principles (replacement, reduction and refinement) of animal research: replacing animals with other methods when available, giving serious consideration to the reduction of animals used, and ensuring that there is ongoing refinement of procedures to enhance animal welfare.

As society increases the attention on natural capital, BMS is pleased to be part of an effort that potentially will contribute to circularity and nature-positive outcomes. We support, and are encouraged by, the recent innovations in the development of synthetic alternatives that could potentially reduce or eliminate the need for LAL reagents in endotoxin testing.

We are working to determine the best path forward and have been actively evaluating current synthetic alternatives, while continuing to fulfill our safety obligations to patients. As a result of our diligent evaluation, we are able to commit to the use of synthetic alternatives to LAL reagents for new medicines in our development pipeline. In addition, we have begun the process of developing the method to transition water testing at our manufacturing sites, which is a large percentage of LAL testing, to synthetic reagents.



Recombinant Evaluation Approach – Points to Consider

- What recombinant reagents are available on the market?
- What consumables (96 well plates, pipette tips etc.) are needed?
- What instrument will be needed to use the recombinant reagent?
- What is compatible to existing equipment/software and reagents?
- Scientific literature and references that evaluate performance of the reagents.



Micro CoE Evaluation Approach

1. Author Recombinant Reagent Evaluation Protocol
2. A minimum of 4 vendors were selected for evaluation.
3. Recombinant reagents were evaluated using water samples from WFI, pre-treatment and USP sites.
4. Each sample was concurrently assessed using traditional LAL
5. At least 3 analysts ran each method.



Key to Data Interpretation

- %PPC – 50-200% would meet the acceptance criterion for USP <85>
- %CV:
 - The lower the %CV value, the more precise the pipetting/analyst technique
 - Acceptance criteria:
 - For traditional LAL and rCR - $\leq 10\%$
 - For rFC- $\leq 25\%$

- What to expect when viewing slides:

%PPC Recovery Graph

Bullets for each assay

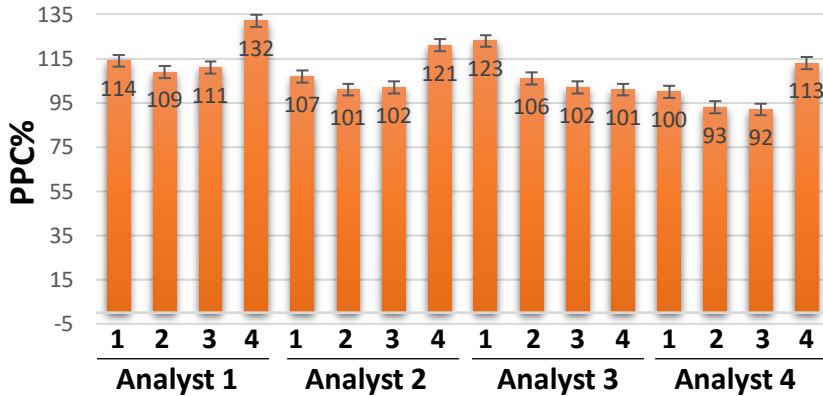
%CV Graph

Analyst Comparison for %PPC and CV%



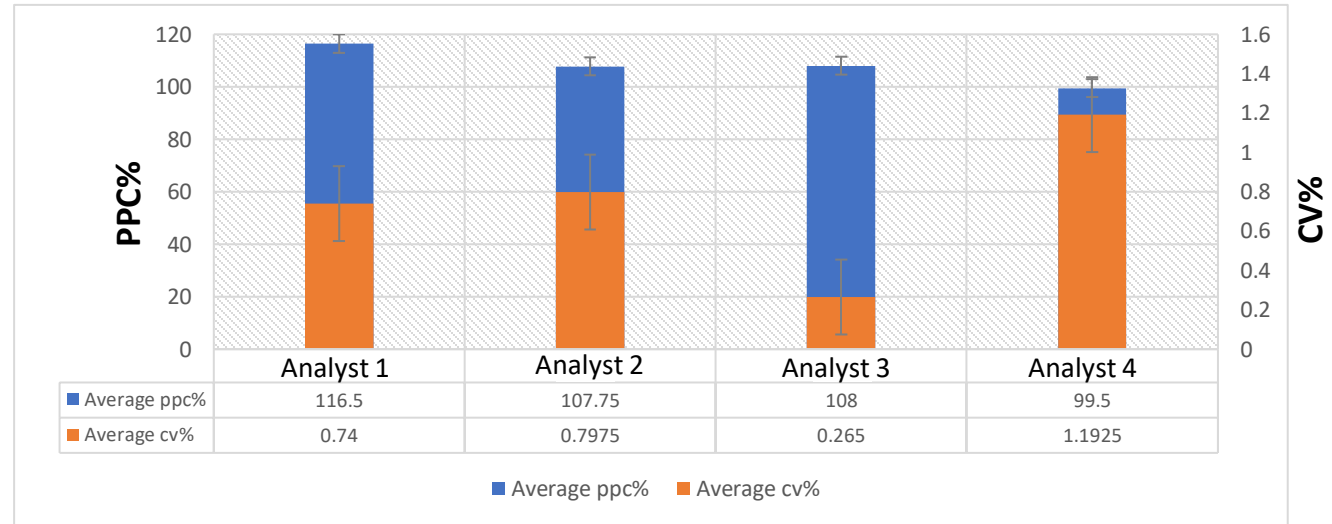
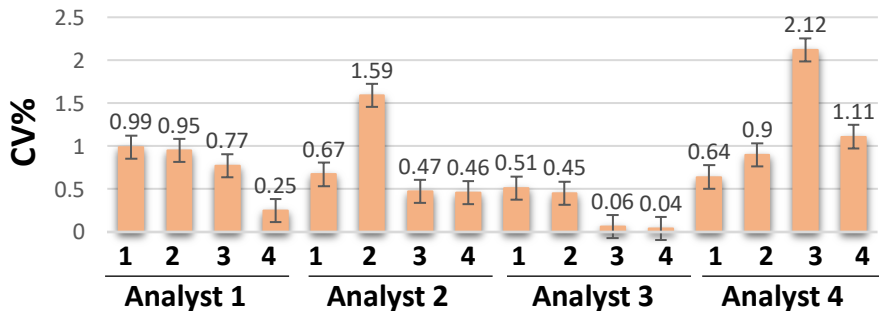
Traditional LAL (Kinetic Turbidimetric Assay)

PPC % Recovery



- Across analysts, PPC% recoveries ranged from 92-132% using the Traditional LAL reagents (kinetic turbidimetric assay).
- All acceptance criteria were met and aligned with USP <85>

CV%





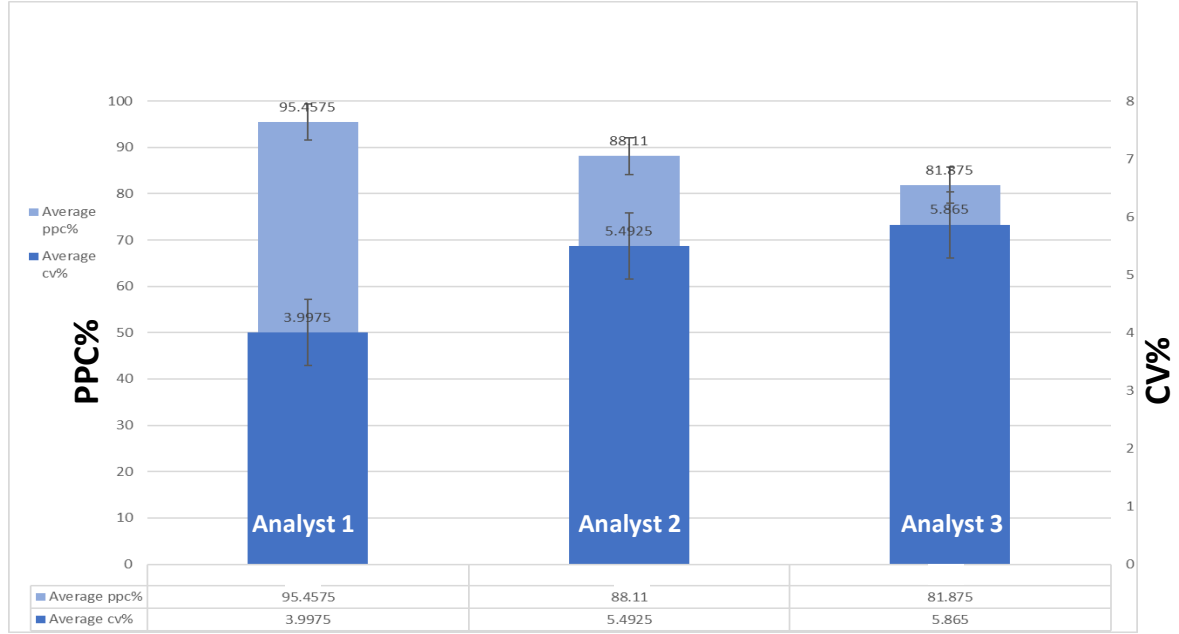
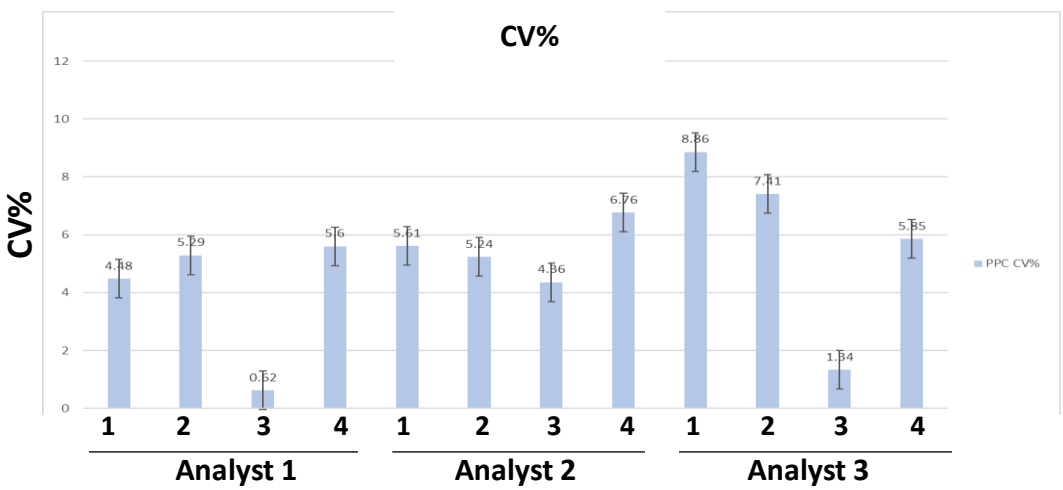
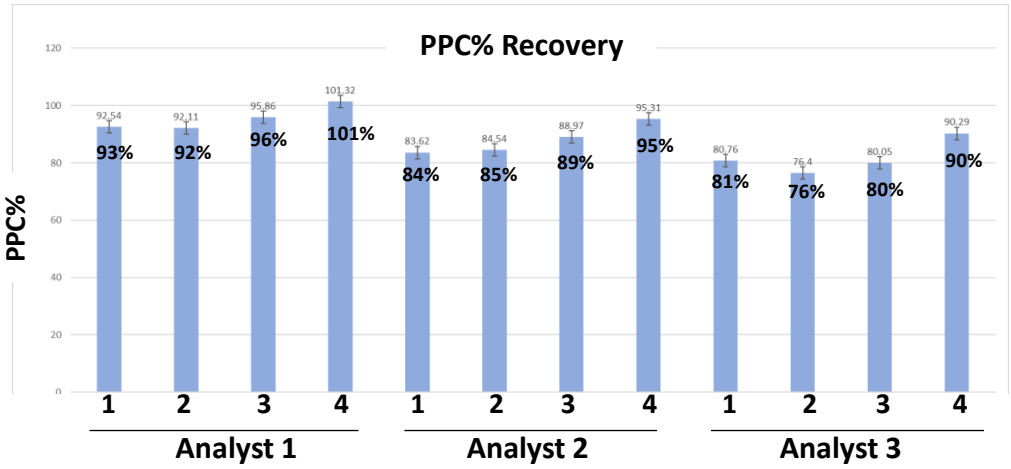
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Vendor 1 - rFC

- Across analysts, PPC% recoveries ranged from 76.4-101.32% using Vendor 1's rFC.
- Reagent consists of a pre-filled plate, which has the standards and PPCs pre-loaded on the 96-well plate.
- Fluorescent-end point readout for rFC assay, yields higher CVs given sensitivity of detection method





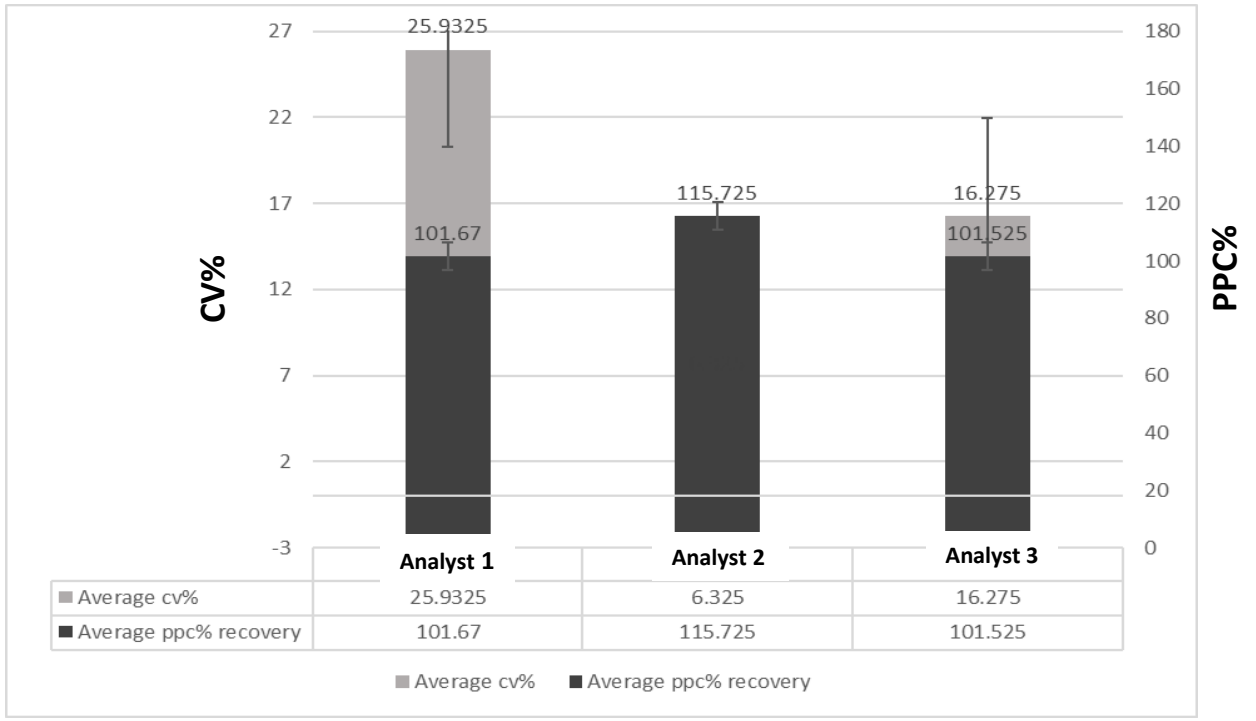
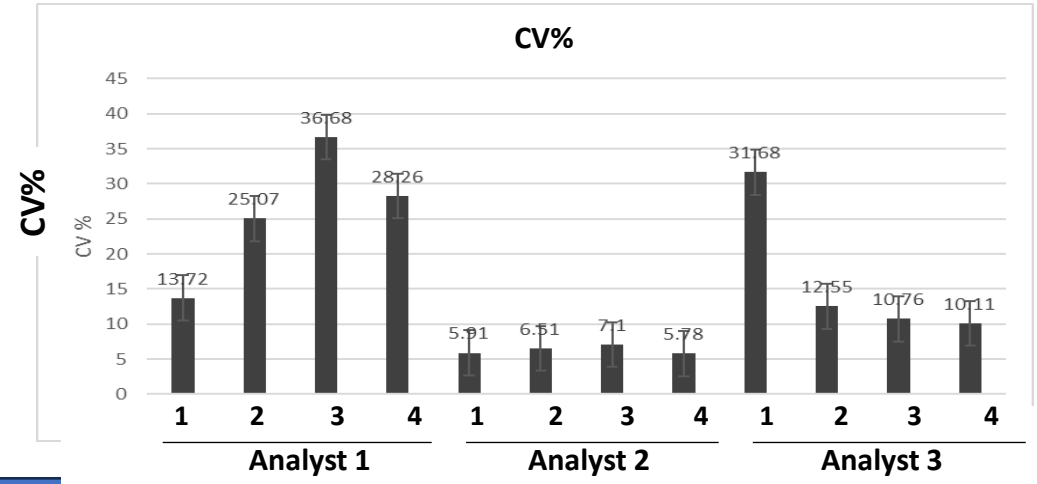
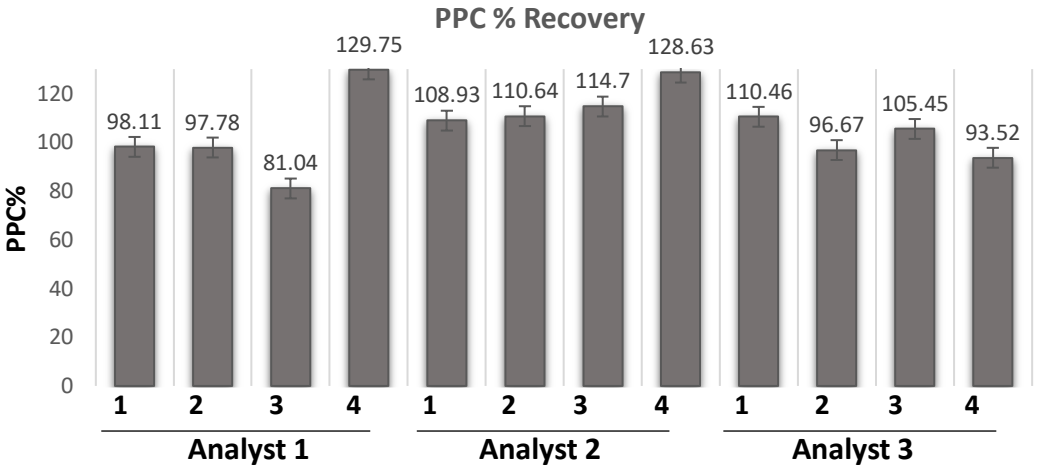
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Vendor 2 - rFC

- Across analysts, PPC% recoveries ranged from 81.04-129.75% using the rFC.
- Fluorescent-end point readout for rFC assay, yields higher CVs given sensitivity of detection method





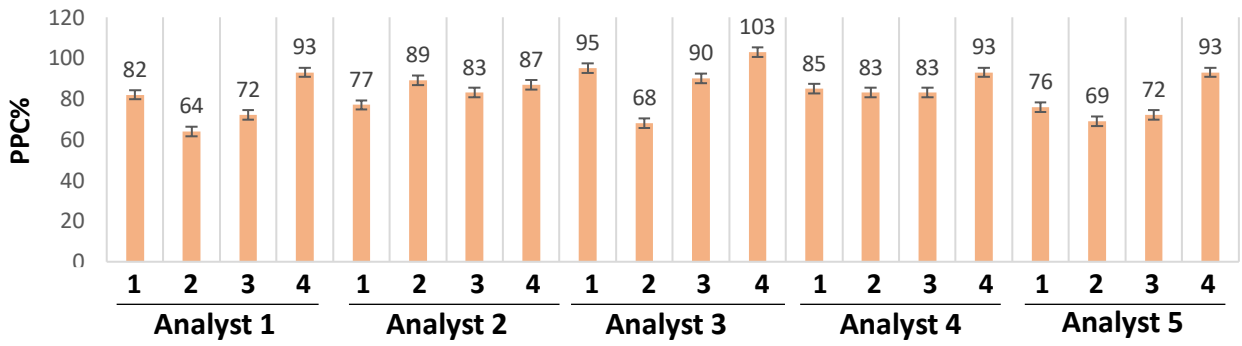
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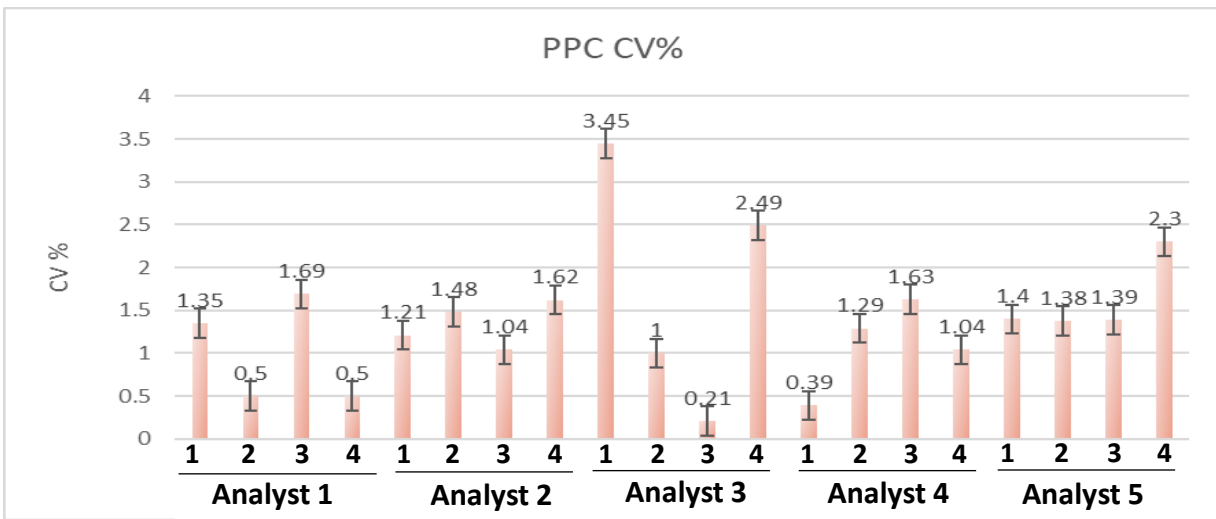


Vendor 3 - rCR

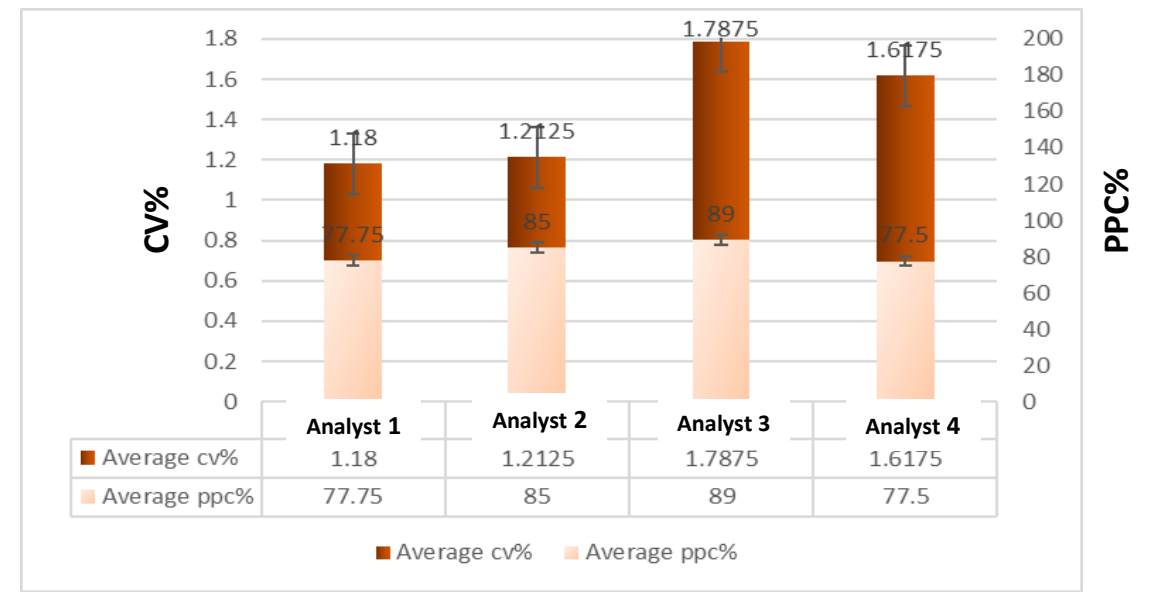
PPC% Recovery



PPC CV%



- PPC% ranged between 64-103%
 - Low PPC recovery compared to other reagents
- Chromogenic readout (yellow coloration)
- Analysts noted that the lysate is yellow after reconstitution, which may lead to low PPC recoveries (more work would be needed to assess)





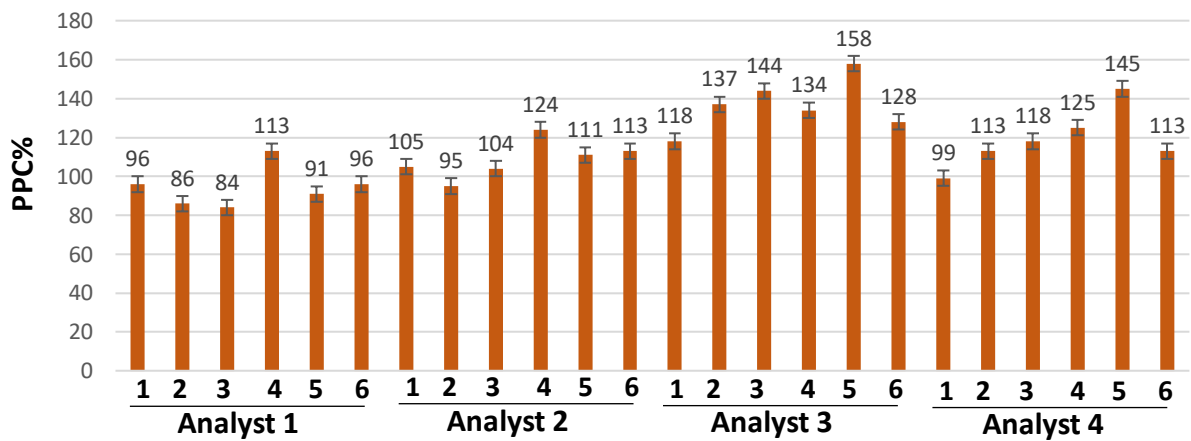
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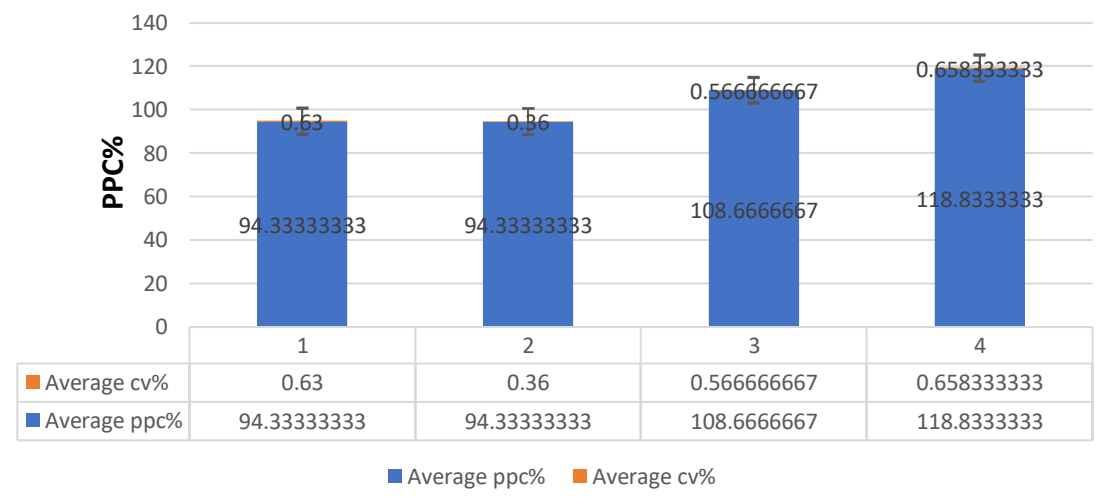
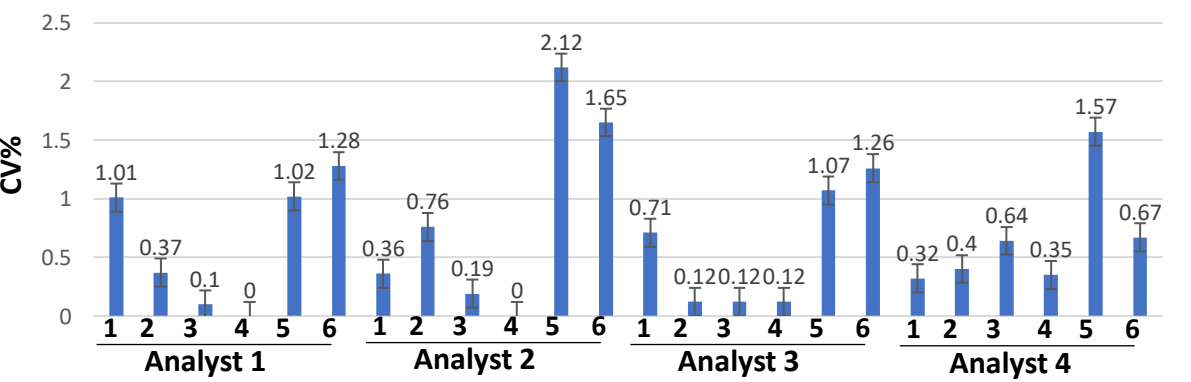
Traditional LAL (Kinetic Turbidimetric Assay) – Evaluation 2

PPC % Recovery



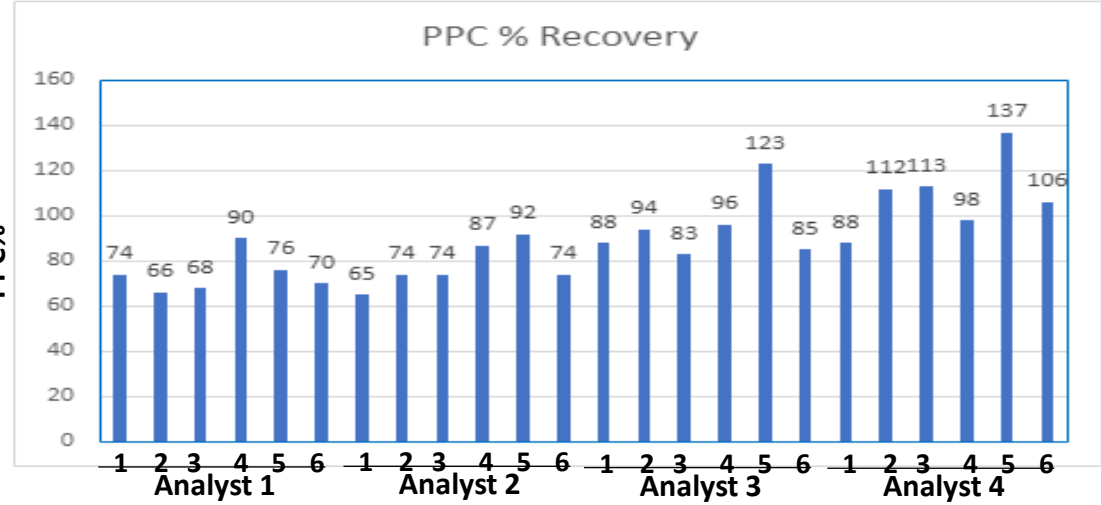
- Across analysts, PPC% recoveries ranged from 84-158% using the Traditional LAL reagents (kinetic turbidimetric assay).
- All acceptance criteria were met and aligned with USP <85>.

CV%

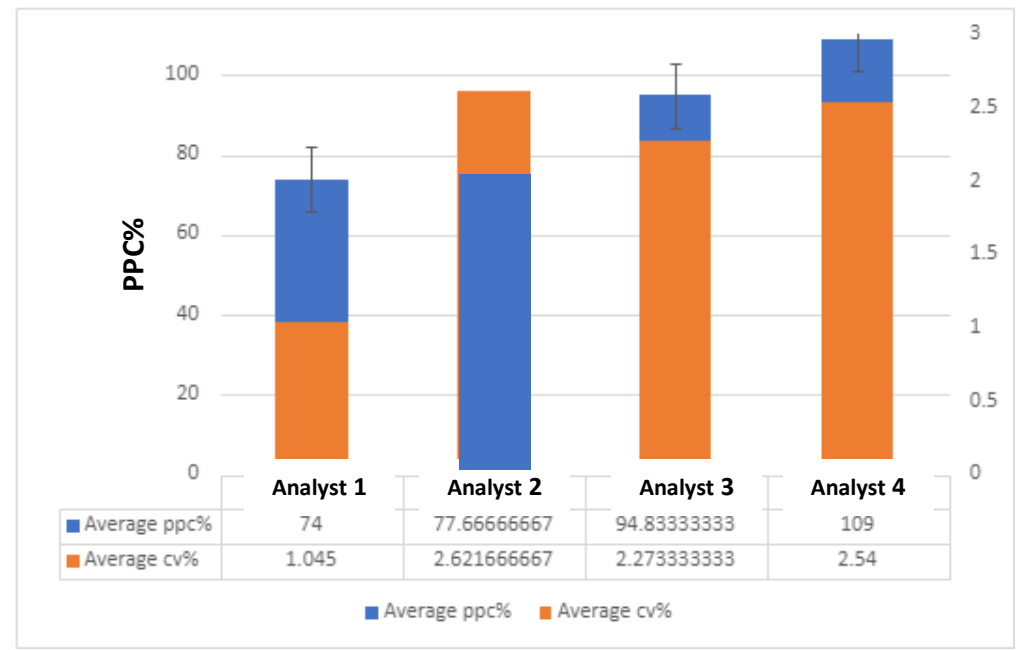
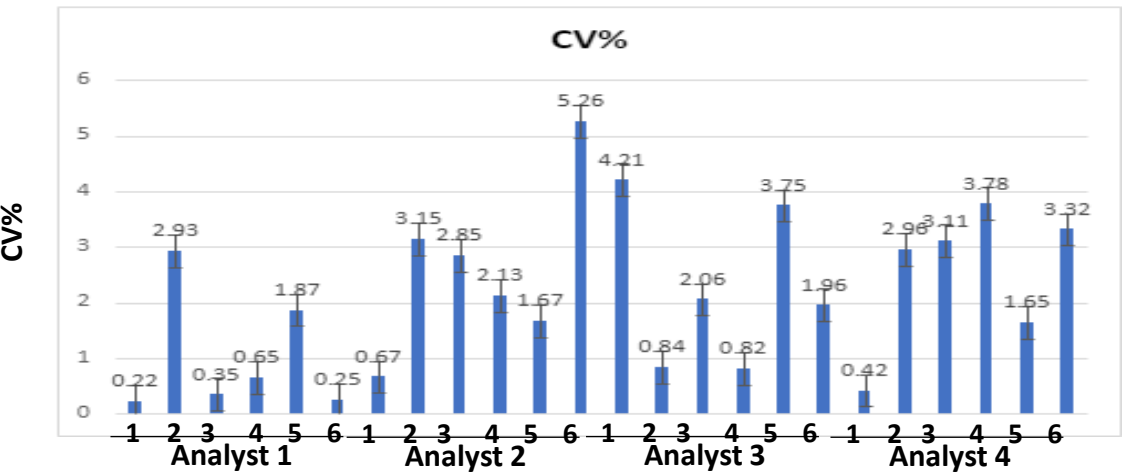




Vendor 4-rCR



- Across analysts, PPC% recoveries ranged from 65-137% using rCR.
- All acceptance criteria were met and aligned with USP <85>.





Conclusion

- Overall, almost all the vendor reagents were comparable to the traditional LAL method for PPC% recoveries.

Trial 1

Parameter	KTA	Recombinant Vendor		
		1	2	3
PPC%	92-132%	76.4-101.32%	81.04-129.75%	64-103%
CV%	0.3-1.2%	0.52-8.86%	5.01-36.68	0.21-3.45%

Trial 2

Parameter	KTA	Recombinant Vendor 4
PPC%	84-158%	65-137%
CV%	0.2-2.12%	0.22-5.26%



Future of rCR and rFC

- The Recombinant Cascade Reagent (rCR) provides operational consistency, as equipment, software, and data output are the same as what is being used across the BMS network.
- Further evaluation of Recombinant Factor C will be conducted as it is comparable to the traditional LAL method.



Take Home Message

- Before making a prompt selection based on convenience, costs, and availability, it is crucial to thoroughly evaluate the reagents available on the market.
- This ensures that the chosen alternative reagent is optimal and is equivalent or better than the traditional LAL methods.