Microbial Contamination and Control Conference

Endotoxin Deviations: A Practical Approach for Laboratory Investigators

PDA®
Parenteral Drug Association
Midwest Chapter

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Eli Lilly and Company

The information to follow are examples from real deviation investigations but all recommendations, mitigation strategies, and points to consider are my own opinion and not that of Eli Lilly & Co.

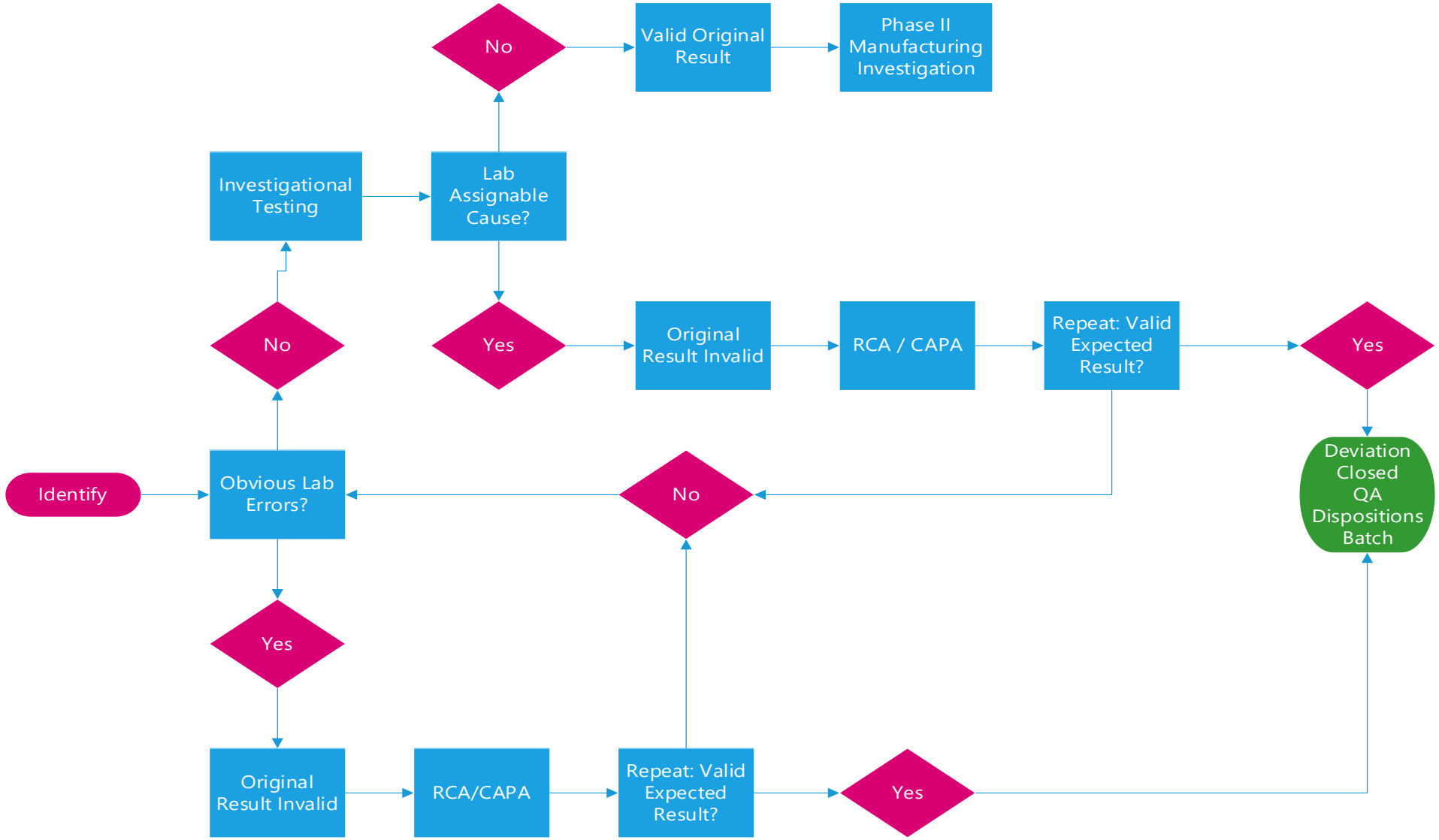
Agenda

1. Background
2. Initial Lab Investigation
3. Investigation Testing
4. OOS Handling & Approach
5. Overcoming Inhibition / Enhancement
6. Case Studies
7. System Suitability Issues & Mitigations
8. Conclusion

PDA Tech Report 88 – Micro Deviations

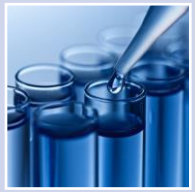
- Phase I – Lab Investigation or Analytical Investigation
  - QC / Lab Management / QA / SMEs
  - Goal – Establish validity of atypical result & determine if lab assignable cause or not
- Phase II – Manufacturing Investigation
  - Sterility Assurance / Ops / Engineering / QA / QC SME / Management
  - Goal – Determine if at any part of the manufacturing process could have led to atypical result

Flow Diagram of Phase I Endotoxin Investigation



*Goal → Determine Validity & Root Cause

Obvious Lab Errors



Sample Preparations

- ❑ Save dilutions until sample release
- ❑ Not knowingly continue a test expected to be invalidated later
- ❑ Right the First Time Culture

Reagents & Consumables

- ❑ Shown to be free of endotoxin & noninterfering
- ❑ Label claim qualification each shipment standards

Instrument

- ❑ Audit trail reviews
- ❑ Roles & responsibilities defined & controlled

Analyst

- ❑ Training & Qualification
- ❑ Analyst interview

Interview

Which question will lead to a better dialogue and nuances of method execution:

“Did you follow sample preparation described in the analytical method?”

“Describe how you executed sample preparation for the analytical method.”

Practice vs Procedure gaps, lack of knowledge or training

Conducting Interviews

- DO
  - ❑ Write questions down
  - ❑ Ask open ended questions
  - ❑ Ask for input from the interviewee
  - ❑ Stimulate back and forth conversation
  - ❑ Start with open ended questions then narrow down to specific yes or no

- DON'T
  - ❑ Ask leading questions
  - ❑ Assume or place blame
  - ❑ Forget to ask for feedback on the process, method, procedure, etc

Investigational Testing for BET

Determine Validity

- Assume results are valid until proven otherwise

Formulate and test hypothesis

- Confirm or discount
- Not repeat – cannot be used as final result

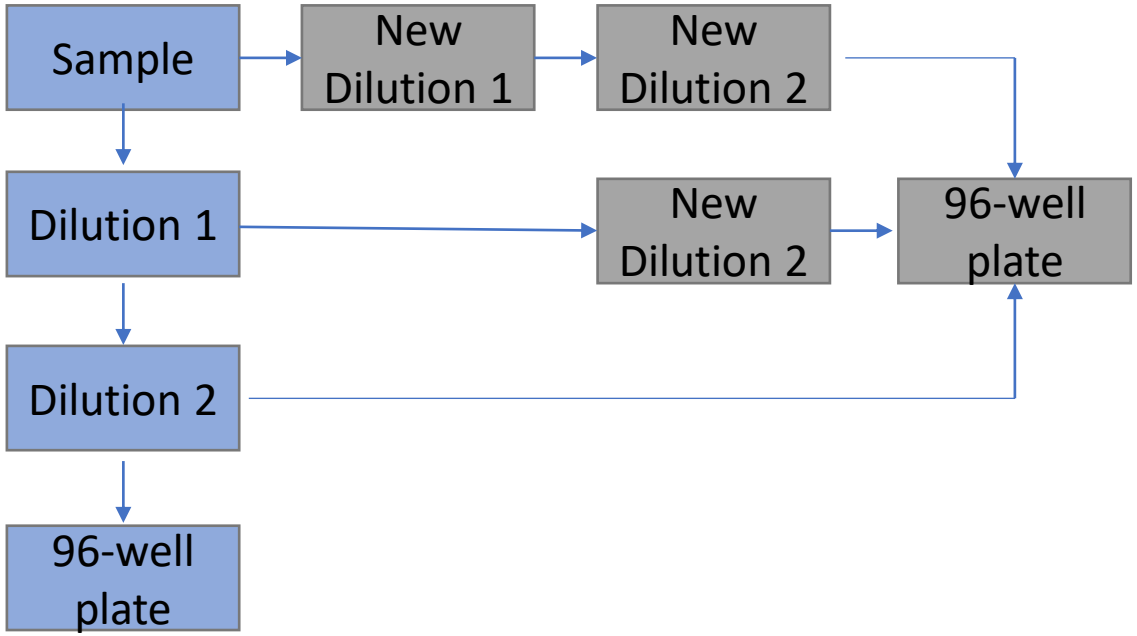
BET

- Testing dilution tubes – contamination introduced during prep
- Repeat of original – 96-well plate suspected or instrument error
- New standard preparation
- New reagent preparation
- pH

Investigational Testing

- Reagents (LRW) → passing Negative Control
- Hypothesis → Contamination during sample prep
- Investigational Testing → Confirm or Disprove Hypothesis
- Outcome → Hypothesis confirmed

Samples	Dilution	Well	Reaction Time (sec)	Average Reaction Time (sec)	Raw EU	Results (Linear Regression) EU/mL	Release Limit
S1	1	A 2	2784	2813	0.0357	0.0357	N/A
		B 2	2841				
PPC	1	C 2	1884	1926	0.141		
		D 2	1967				
	PPC Value: 0.1	% PPC Recovery :		105%	(PPC - SAMPLE 1) Endotoxin Recovered : 0.105		



Outcome	
New Dilution 2 from Original Sample	<0.0100 EU/mL
New Dilution 2 from Original Dilution 1	0.0282 EU/mL
Original Dilution 2	0.0230 EU/mL

Out of Specification Handling & Approach

Establish Validity

- Even invalid OOS should be rigorously investigated

Cannot invalidate without lab assignable cause

- 5x Retest

Regulatory Experience

- Provide list of valid and invalid OOS investigations

OOS Investigational Testing

7 excipient samples with endotoxin activity resulting in an OOS event

Obvious lab errors	Reagents	Investigational Testing	Isolated Event?
Sample preparation	Negative Controls	50mM Buffer → <0.01EU/mL	Vials before and after event
Consumables	Common reagent	Dispersing Reagent Vial → 0.13EU/mL	Vendor inquiry
Interview	50/50 v/v Dispersing/Buffer	0.5% Dispersing Reagent → 0.12EU/mL	Analyst coaching
		50/50 Dispersing/Buffer → 0.05EU/mL	Switch lots

Overcoming Inhibition / Enhancement

- pH adjustment
- Buffers
- Dispersing agents
- Mixing
- MVD

MVD

- MVD = (endotoxin limit x sample concentration) / λ
- Guidance in <85>, <1085>, proposed <86>
  - Dilute to MVD
- Pooled samples adjustment
  - For example: MVD is 1500 but 3 vials are pooled → 1/3 MVD is 500

Material of Construction

Regulatory focus during inspections

Guidance from 2012 FDA Testing Questions & Answers Document

Established Hold Times

PETG, PS, PE

EVA, ULDPE

Do not use PP

QC Labs need to be looped in manufacturing changes impacting sample containers

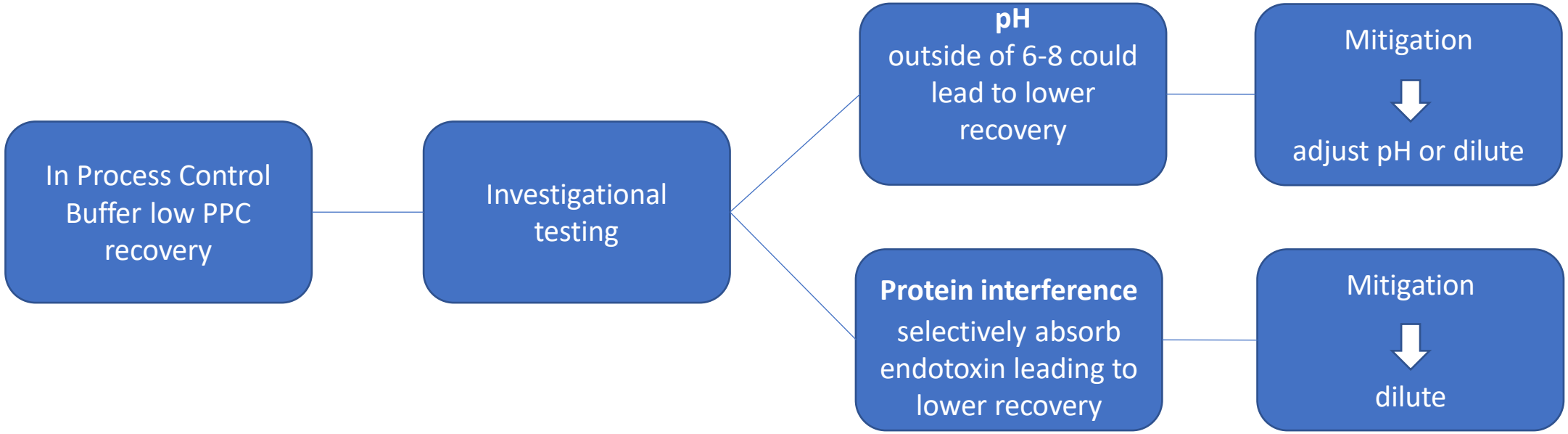
LER

Included in BLA submissions

Focus on matrix interaction

i.e. chelator and surfactant in the matrix

Low PPC Deviation Investigation



- pH: 7.52 → within recommended range
- UV analysis → positive for protein content
  - Original sample was diluted 1:10
  - IPC DP requires 1:100

- Manufacturing Investigation (Phase II)
  - Confirmed sample pulled from wrong tank

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Low PPC Deviation Investigation

Original Verification

- Single supplier of rFC Reagent
- Diluent – MgCl2

Low PPC Recovery

- Secondary supplier utilized
- On going trend with material

Follow up method development activities

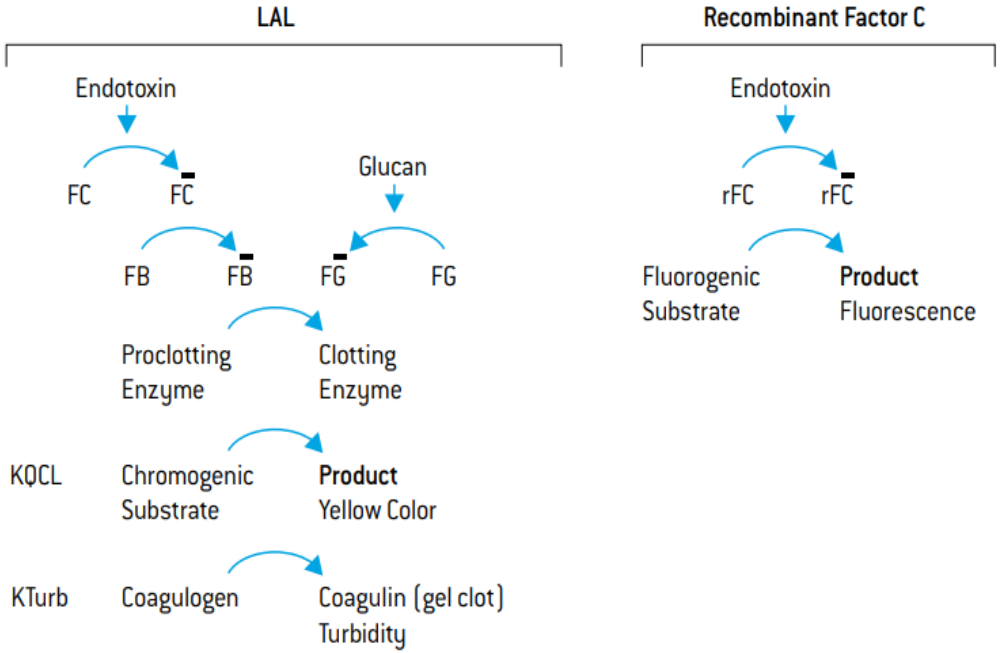
- Additional testing confirmed MgCl2 interference with 2nd supplier
- Updated diluent and dilution scheme to work with both suppliers
- Tris Buffer

Supplier	Avg. PPC Rec (%)	Number of Samples
Supplier 1 Verification	97	3
Supplier 1	96	17
Supplier 2	51	4
Supplier 2 Post Verification Update	91	19

2nd supplier verification on all commercialized molecules utilizing rFC platform

Beta Glucan Interference

Beta glucan interference
- Activate factor G pathway, false positive
- cellulose filter in the manufacturing process
- raw bulk materials (yeastolate)



*Courtesy of Lonza package insert.

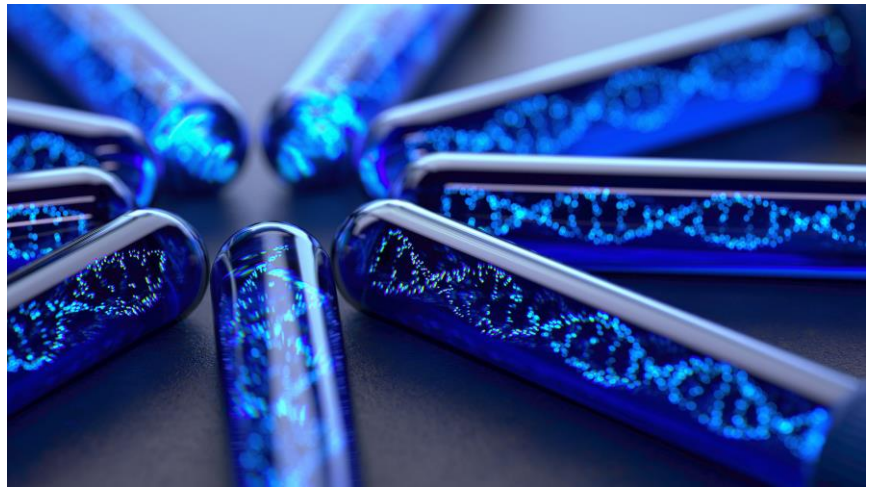
Recombinant assays mitigate beta glucan false positive interference in LAL assays

Supplier	Reagent	Sample	Result (EU/mg)	%PPC Recovery
Supplier 2	LAL	Yeastolate	0.0687	133
Supplier 3	LAL		>4.00	N/A
Supplier 6	LAL		0.0639	123
Supplier 1	Recombinant		<0.0400	79
Supplier 2	Recombinant		<0.0400	71
Supplier 3	Recombinant		<0.0400	100
	Recombinant		<0.0400	87
Supplier 4	Recombinant		<0.0400	56
Supplier 5	Recombinant		<0.0400	76
	Recombinant		<0.0400	91
Supplier 6	Recombinant		<0.0200	107

USP <86> if approved, early adoption in Nov 2024

Recombinant chapter

- rFC – end point florescence
- rCR – chromogenic, absorbance



Compendia Impact

- PhEur - Replacing RPT with MAT – 2026
- Could LAL be next?

Case Study #1 – Cleaning Validation Samples

Background

- Submitted as WFI
- Analyzed on alternate rFC platform
- Controls passed
- Rinse samples inhibited

Impact

- Multiple deviations
- Manufacturing equipment

Method
LAL comparison
Validation
Man
Training
Technique
Performance History
Instrument PM
Pipettes
Equipment
Reagents / Consumables
Standards
Material
pH
MoC / Storage
Chemical properties
CV Cleaning Cyle
Sample

Initial Analytical Investigation

- pH of sample + rFC reagent
- pH of control & rinse sample
- Normal results:
  - Particulates
  - Bioburden
  - Phosphate
  - Conductivity
- Business Continuity Plan - LAL

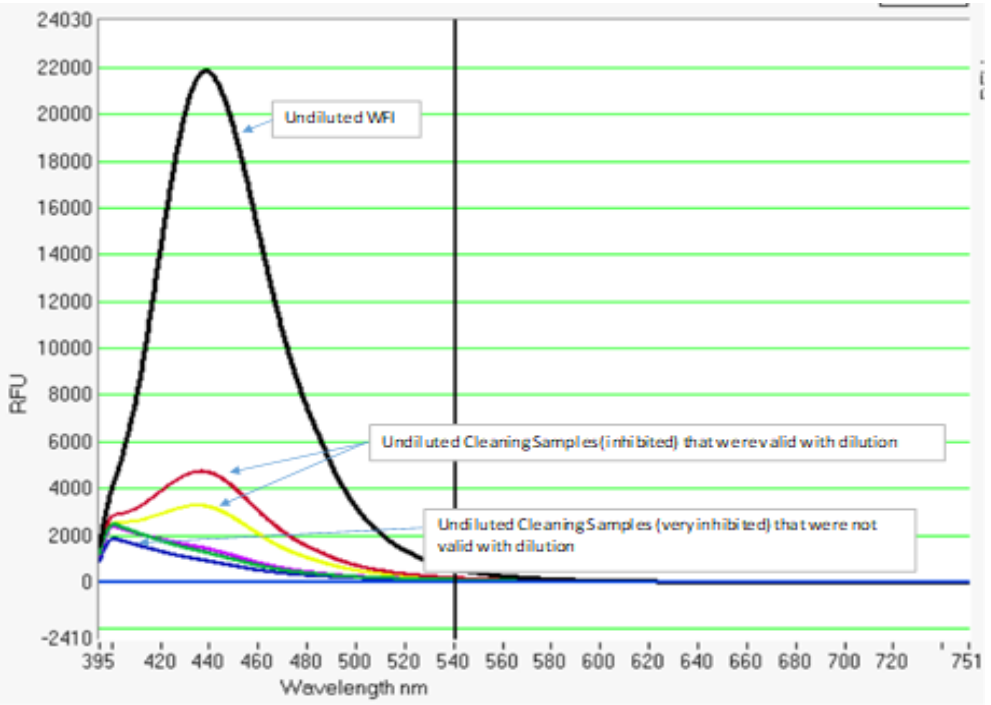
Detailed Investigation

Assumption was rinse sample is equivalent to WFI

- Residual product or cleaning reagent?
- Cleaning cycle passed indicating the appropriate removal of cleaning agent and any residual product

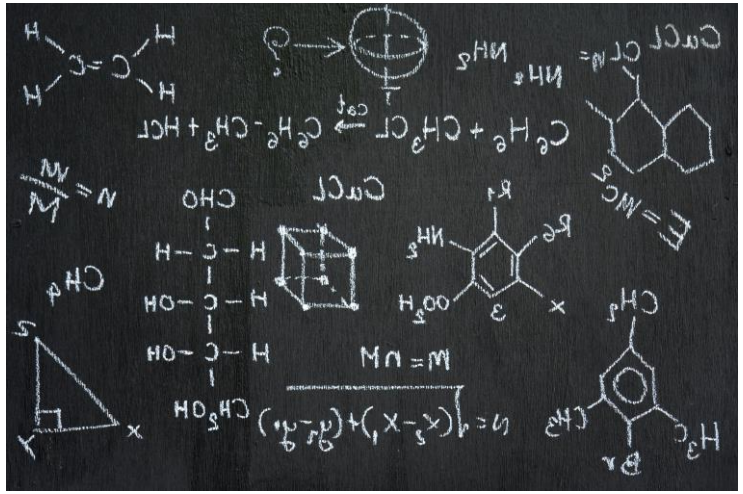
Investigation testing

- Spectral scans: submitted cleaning samples ≠ WFI
- Trace amounts of CIP (surfactant) inhibit PPC recovery on rFC and not LAL
- Certain reagent suppliers more sensitive to interference than others



CAPA

- Method Development
- Harmonized dilution scheme



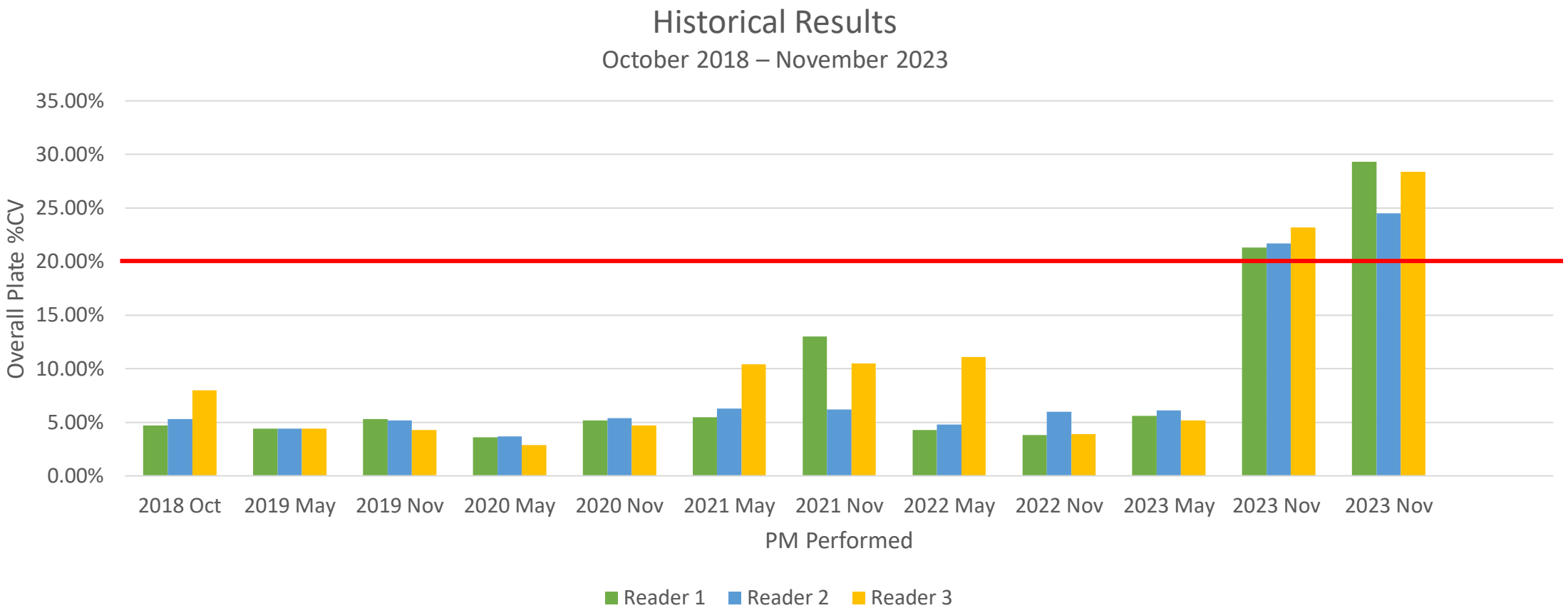
Learning

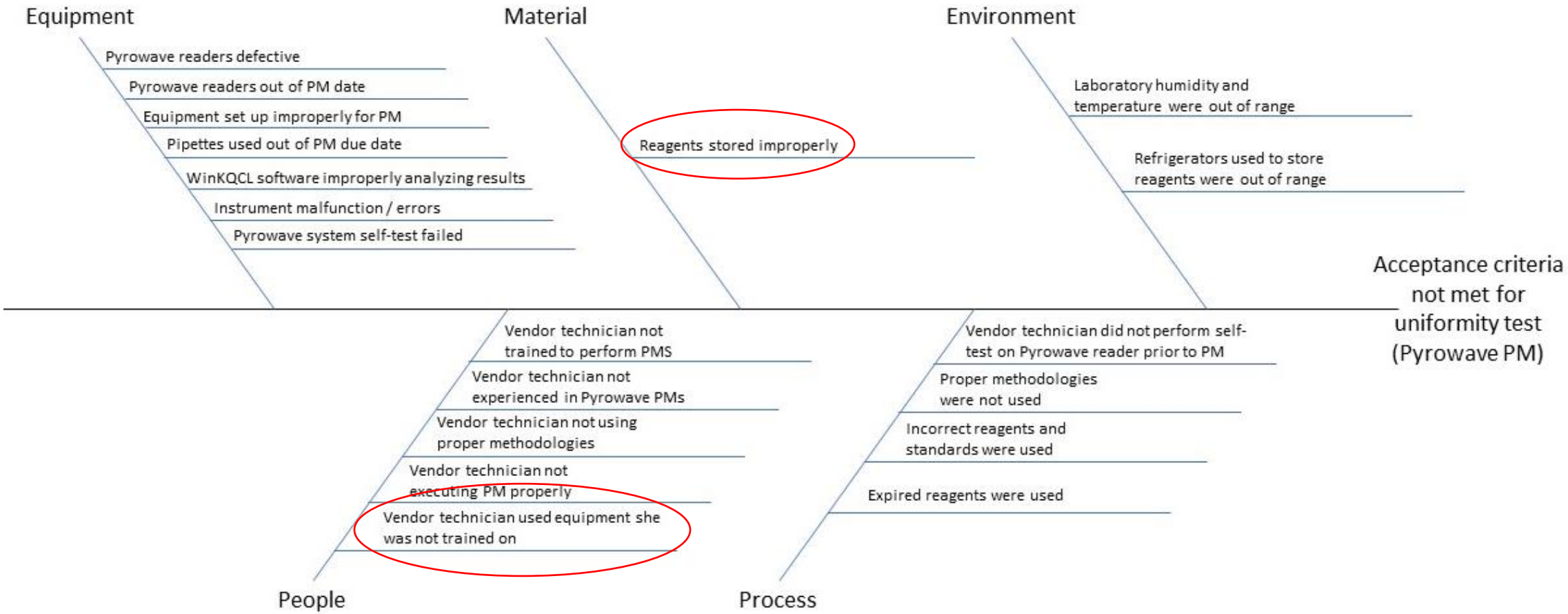
- Consider all impacts when evaluating process change or implementing new methodologies / technologies
- Method was optimized for WFI, Clean Steam
- Assumption was rinse water = WFI

Case Study #2 – Plate Reader PM Failure

Background & Impact

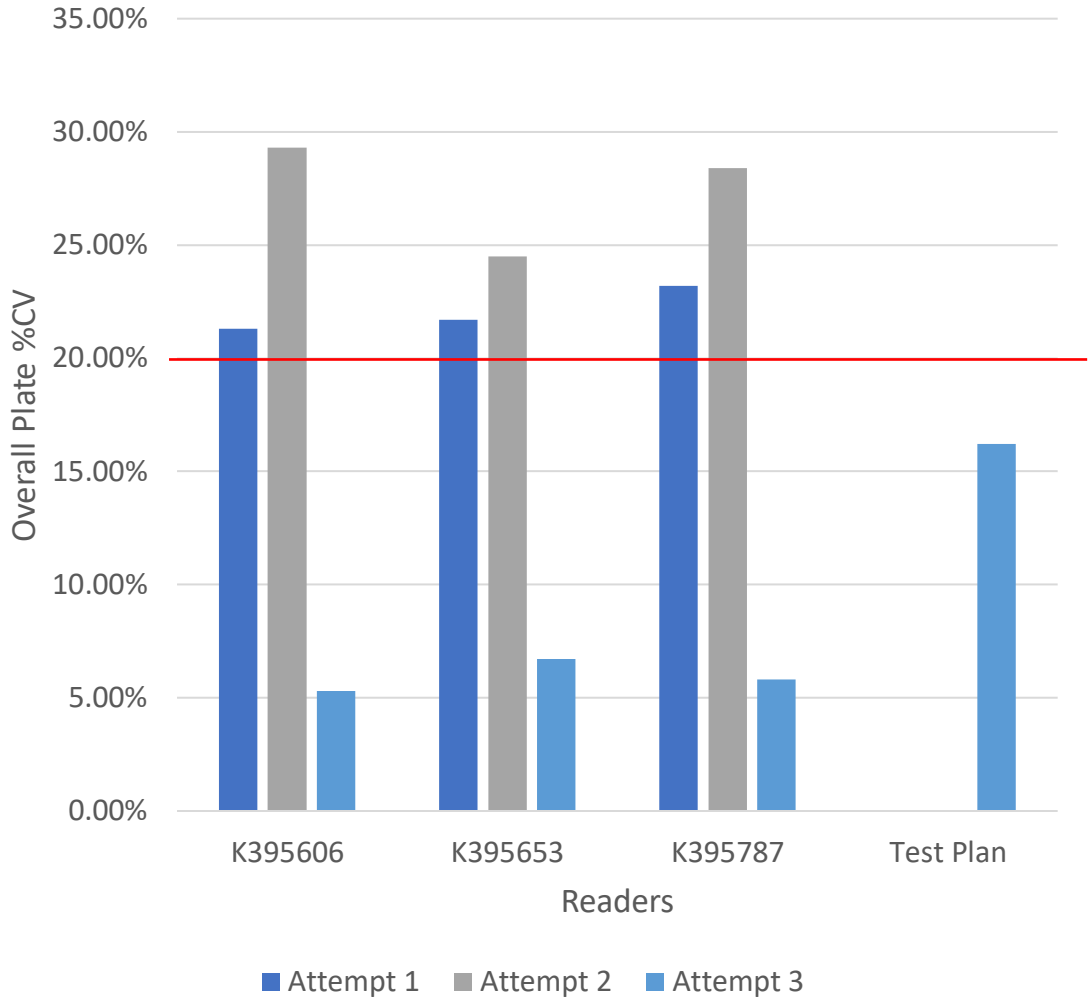
- Vendor PM
- Uniformity on Fluorescence Readers
- Absorbance Readers
- “Tagged Out” Plate Readers


Historical Results
October 2018 – November 2023
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Overall Plate %CV
2018 Oct
2019 May
2019 Nov
2020 May
2020 Nov
2021 May
2021 Nov
2022 May
2022 Nov
2023 May
2023 Nov
2023 Nov
PM Performed
Reader 1
Reader 2
Reader 3


Equipment
Pyrowave readers defective
Pyrowave readers out of PM date
Equipment set up improperly for PM
Pipettes used out of PM due date
WinKQCL software improperly analyzing results
Instrument malfunction / errors
Pyrowave system self-test failed
Material
Reagents stored improperly
Environment
Laboratory humidity and temperature were out of range
Refrigerators used to store reagents were out of range
Acceptance criteria not met for uniformity test (Pyrowave PM)
People
Vendor technician not trained to perform PMS
Vendor technician not experienced in Pyrowave PMs
Vendor technician not using proper methodologies
Vendor technician not executing PM properly
Vendor technician used equipment she was not trained on
Process
Vendor technician did not perform self-test on Pyrowave reader prior to PM
Proper methodologies were not used
Incorrect reagents and standards were used
Expired reagents were used

Investigational Testing

- Properly stored reagents
  - %CV = 16.2%
- Familiar Pipettes
  - Attempt 3

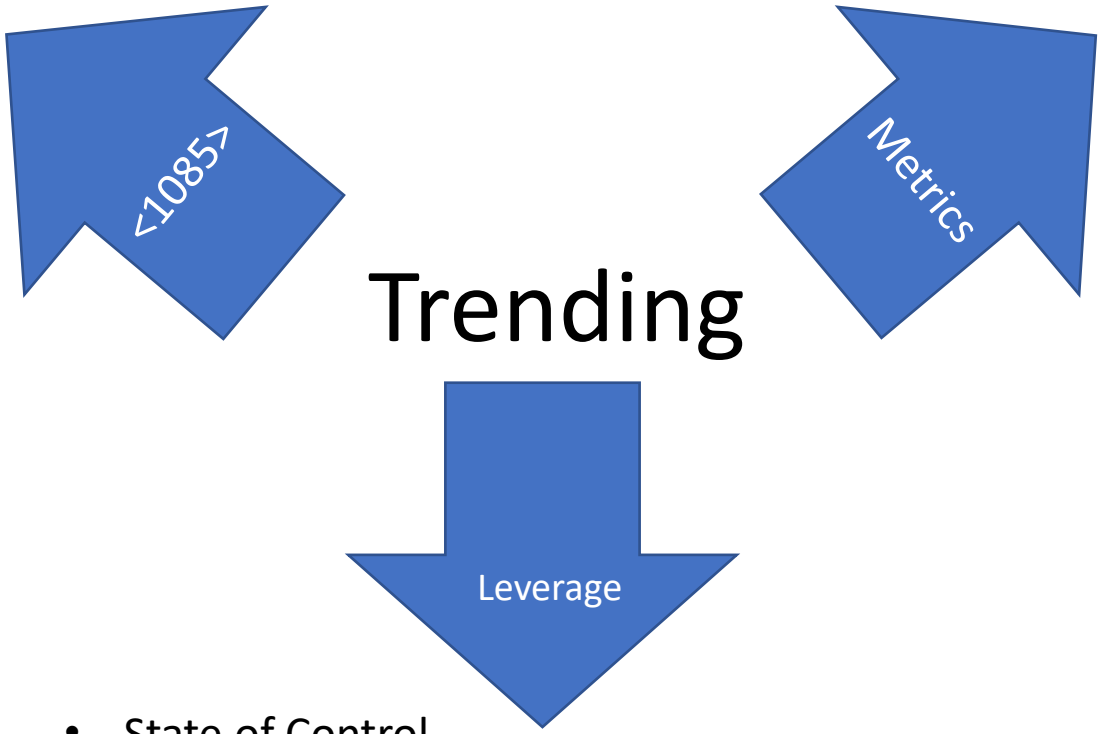


CAPA & Summary

Impact:	• Plate Reader PM Uniformity Test failures on all 3 Micro QC lab Readers • 2 weeks
Root Causes:	• Improper storage of reagents • Vendor technician using equipment without training
CAPA:	• Procedural updates • Network Shared Learning

System Suitability Issues

"Invalid test should be tracked and trended to look for patterns and trends that might require a corrective action"



- Invalid assay rate
- Invalid sample rate
- %PPC Recovery
- %CV for PPC & Standard wells

How does this look for our lab?

- Document every system & sample control issue
- Monthly tracking of metrics
- Upper control limit established

- State of Control
- Support repeat testing without Deviation record

Mitigation Considerations

Training

- Robust Training Program
- Observation & Hands on
- Intervention for identified trends

Ready to Use Plates

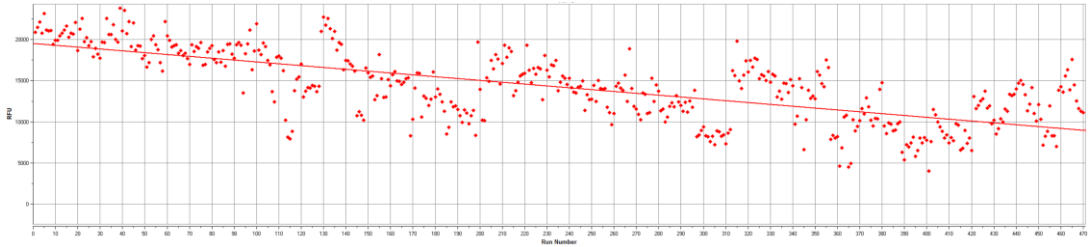
- Minimize analyst technique issues & pipette variations at small volumes

Reagent & Standards

- Monitoring standard signal / response (end point fluorescence) & reaction times (Kinetic LAL)

Instrument Optimization (Fluorescent Readers)

- Scan rate
- Sensitivity / Gain
  - Utilize entire dynamic range



Conclusion

- Obvious Lab Errors
  - Sample Prep
  - Instrument
  - Analyst
- Investigation Testing
  - Test Hypothesis
  - Not a Retest or Repeat
- Recombinant Technology
  - USP <86>
- Case Studies
  - Method Root Cause
  - Human Root Cause
- Tracking and Trending
  - USP <1085>

Questions?