Baxter

Invalid Trending in Bacterial Endotoxin Testing (BET)

From Compliance Event to Sustainable Control

Sean Toler

Sr. Technical Consultant, Quality

Why are we talking about invalid trending?

Context of the Case Study

FDA Enforcement Action 2022 / 2023

- FDA inspections at one of our facilities led to observations on a form 483 related to BET OOS Investigations.
  - Facility in question **incorrectly interpreted system/sample suitability parameter failures** (i.e. %CV of standard dilution excursion, %CV PPC excursion, blank reaction, etc.) as OOS
  - Observation was related to poor investigations defaulting to **“particulate contamination”** or **“pipetting error”** without evidence of effective corrective actions

Company Response

- Review all BET-related OOS and Invalid Events over a **5-year period** and implement corrective actions as necessary

Outcome

- The comprehensive review showed **no endotoxin risk**
- Identified issues centered on **terminology misunderstanding**
- During the review there was a **lack of practical industry guidance for trending invalid results.**
- **Statistical process control was critical to assessing the program**

Manufacturing Site Details

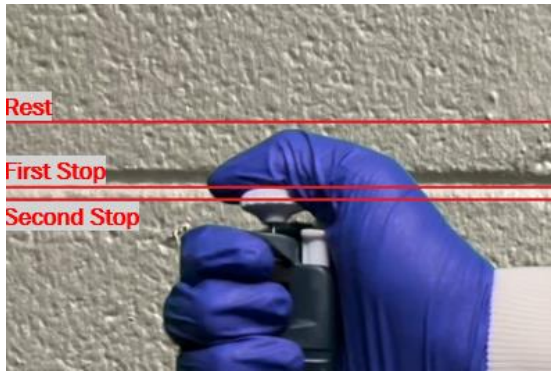
- Premix Drug Solution in Glass Container
  - Vial
  - Ampoule
- Terminal Sterilization: Overkill approach
- BET primarily performed via kinetic turbidimetric method
  - 96-well plate, spectrophotometer
  - Some products require Gel Clot method
- Ahmedabad, India

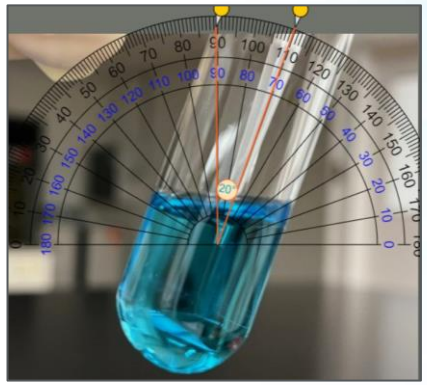
What we actually found

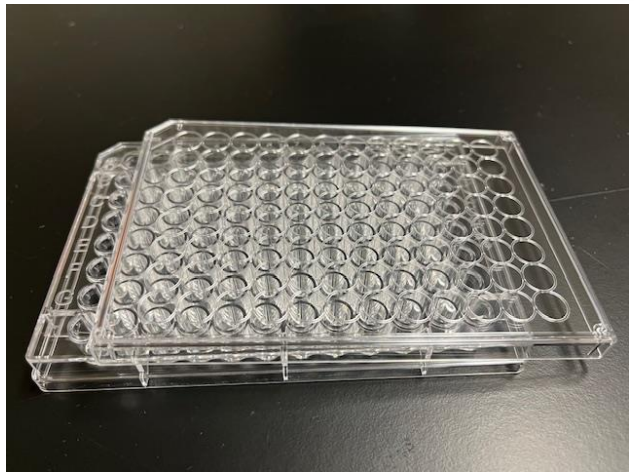
No systemic endotoxin risk across the program BUT…

There was plenty of room to improve (preventative) the program:

- Strengthen Technical Competency
  - On the Job Training Improvements
  - Instructor-led Technical Training for BET
  - Improve aseptic techniques
- Equipment Performance
  - Enhanced preventive maintenance
  - Laboratory infrastructure updates
- Modernize Investigation and Trending Practices
  - Implementation of invalid result trending program
  - Expanded oversight with corporate SMEs and 3rd party reviews
  - Harmonize process for invalid results







The Terminology Problem: Invalid vs. OOS

USP <1085>

- Clearly differentiates invalid tests from OOS
- Implication from USP <1085> is that not every invalid result requires an investigation
- Invalid results need to be tracked and trended to identify potential improvements

Manufacturing Site's Misinterpretation

- Leads to unnecessary "OOS" investigations
- Confuses the inspector
  - Belief was that the inspector thought the plant had an inordinate number of samples that were "invalidated" due to laboratory error during multiple OOS investigations
  - The misunderstanding resulted in a subsequent Warning Letter due to the criticality of the endotoxin's impact on product safety

Poll Question

For your BET laboratory, what invalid rate do you typically expect?

A. – Less than 1%

B. – Anywhere from 1% to 3%

C. – 5% to 10%

D. – Greater than 10%

E. – All of the above

F. – Do not track this metric

The Terminology Problem: Invalid vs. OOS

Invalid results are triggered by system suitability or sample suitability :

- Biological variability of LAL reagents
- Consumable variability
- Analyst pipetting precision
- Dependence on solution / product in test

Wide Inter-Lab Variability

- Every laboratory is different (products, consumables, personnel, lot to lot variability, etc.)
- Wide range for invalid rate (1% to 10%... Maybe even 20%)
- Cross-laboratory comparisons are meaning less

Baxter

Statistical Process Control

Principles are ideal for BET invalid trends

Statistical Process Control (SPC)

SPC Compares “Like for Like”

- Literature review indicated strongly emphasizes that the BET invalid rate of a particular laboratory should only be compared to historical performance of that laboratory and not compared with others.
- SPC uses only your historical data to establish the performance baseline
- There is not a published “good” and “bad” invalid rate.
- Avoids cross-laboratory variability
- Tailored to inherent variability to BET methods
- Accommodates complexity by evaluating stability over time rather than one-off invalid results
- Invalid results are tough (in most cases impossible) to find definitive root cause
- Focuses on trends as recommended by USP <1085>
- Easy to understand and identify control thresholds
- Platform agnostic

SPC Framework

1) Invalid sample rate

- Number of invalid **samples** / total samples

2) Monthly rate (include mean)

3) Control limits: set on previous year data

- 1σ (·····)
- 2σ (- - - - -)
- 3σ (– – – – –)[UCL]

4) Upper levels of particular importance

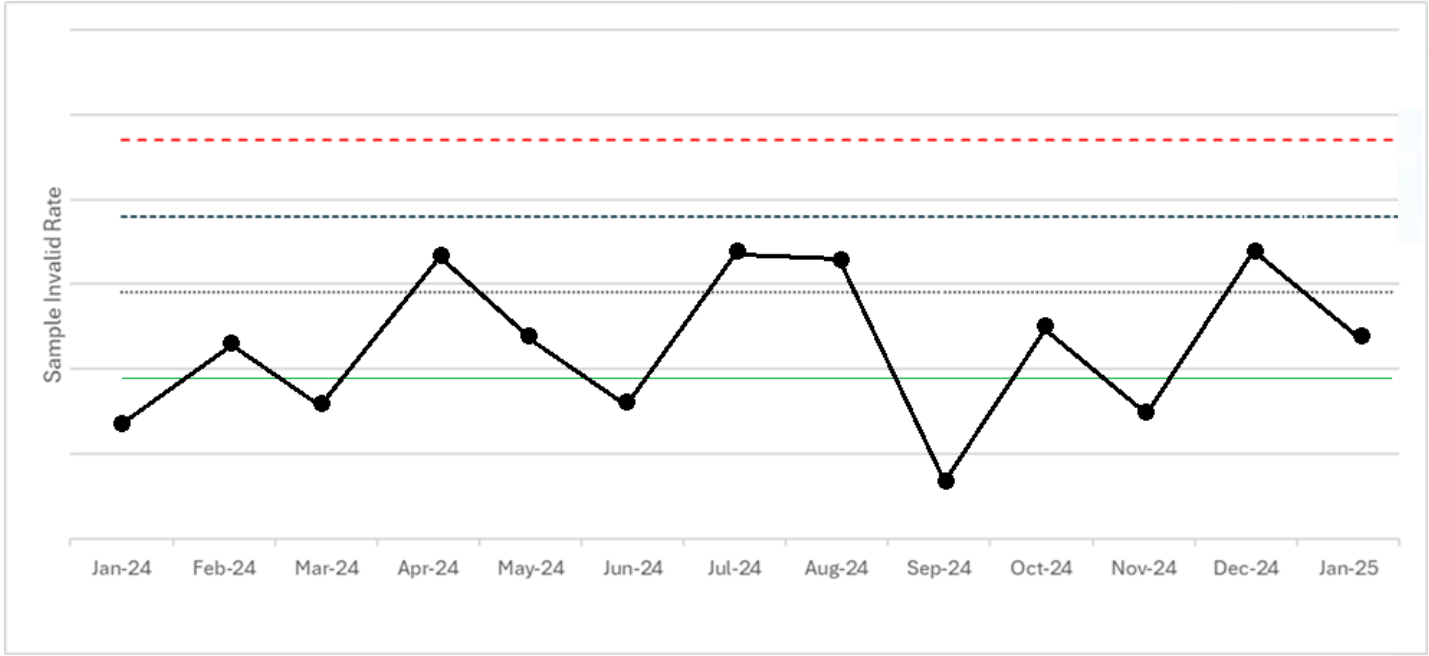
5) Chart for an entire year (13 data points)

6) Signal Analysis

- Nelson rules

7) Action Threshold

- Investigate rule violations



SPC Framework: Nelson's Rules

There are more rules than this, this was what we decided to use

1) A single point exceeds the 3σ Level (UCL)

2) Two (2) out of three (3) points in a row exceeds the 2σ Level

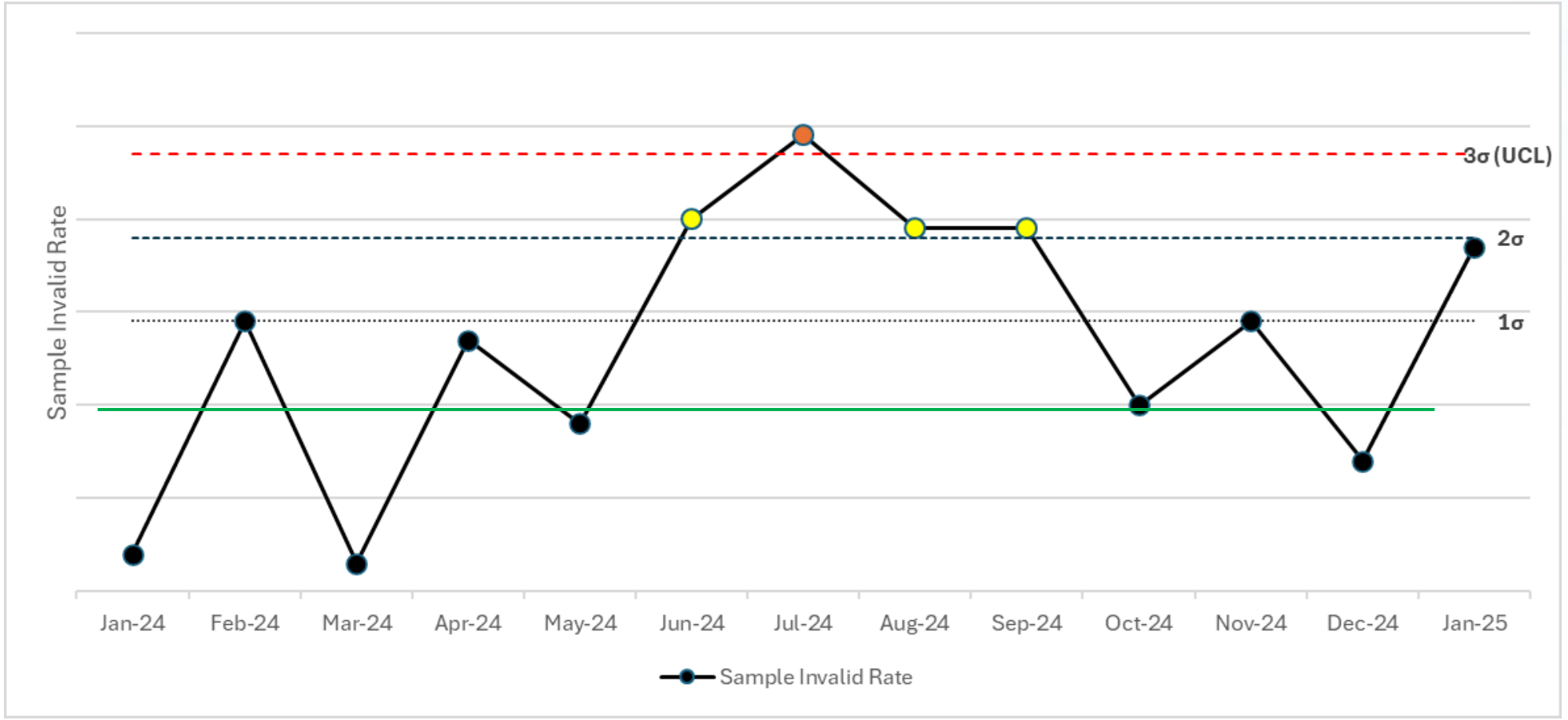
3) Four (4) out of five (5) points in a row exceeds the 1σ Level

4) Six (6) or more points in a row that are continually increasing

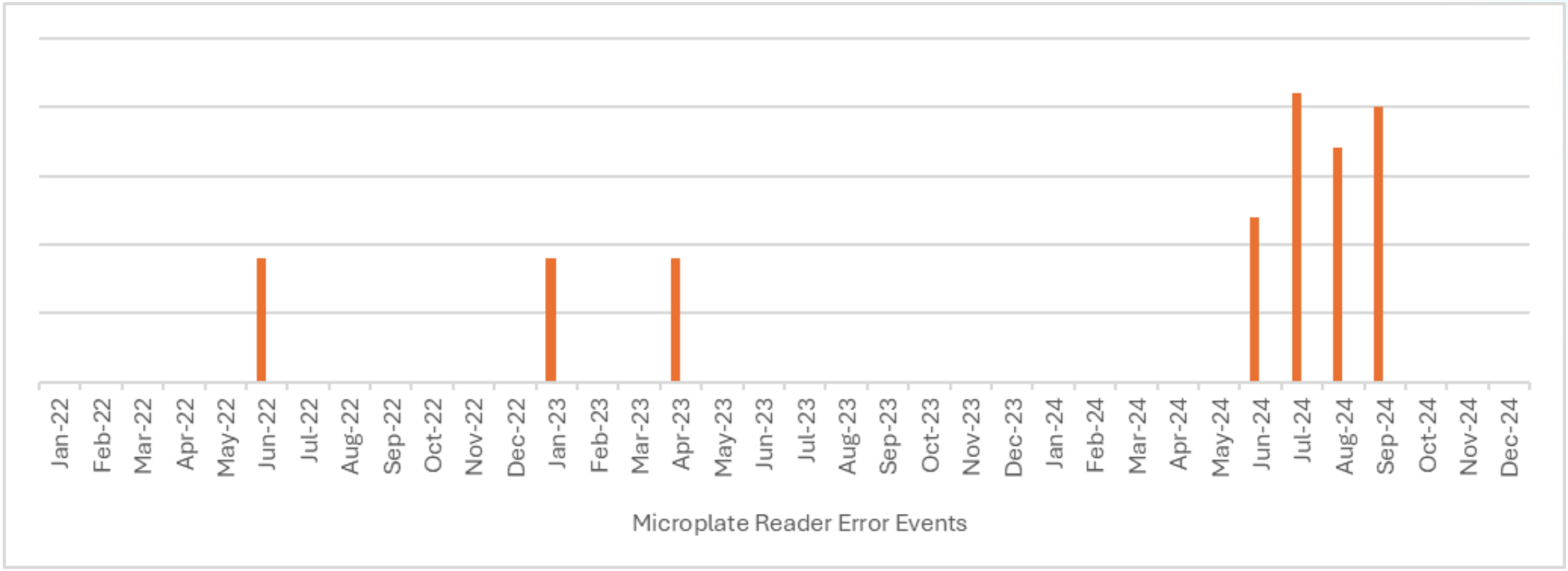
5) Eight (8) or more in a row without a single point within the mean and 1σ level on either side

6) Nine (9) or more points in a row that are on the same side of the mean

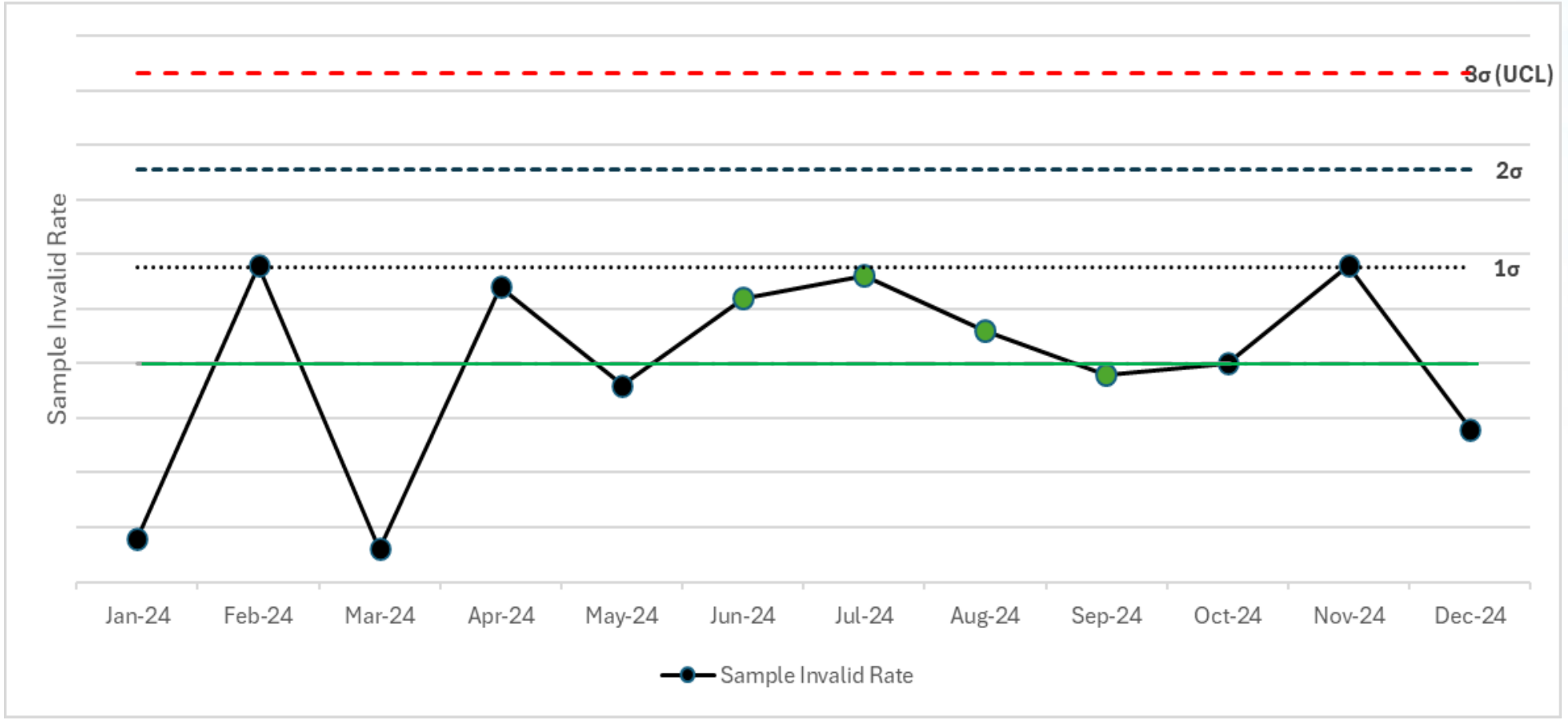
Case Study: Patterns - 1



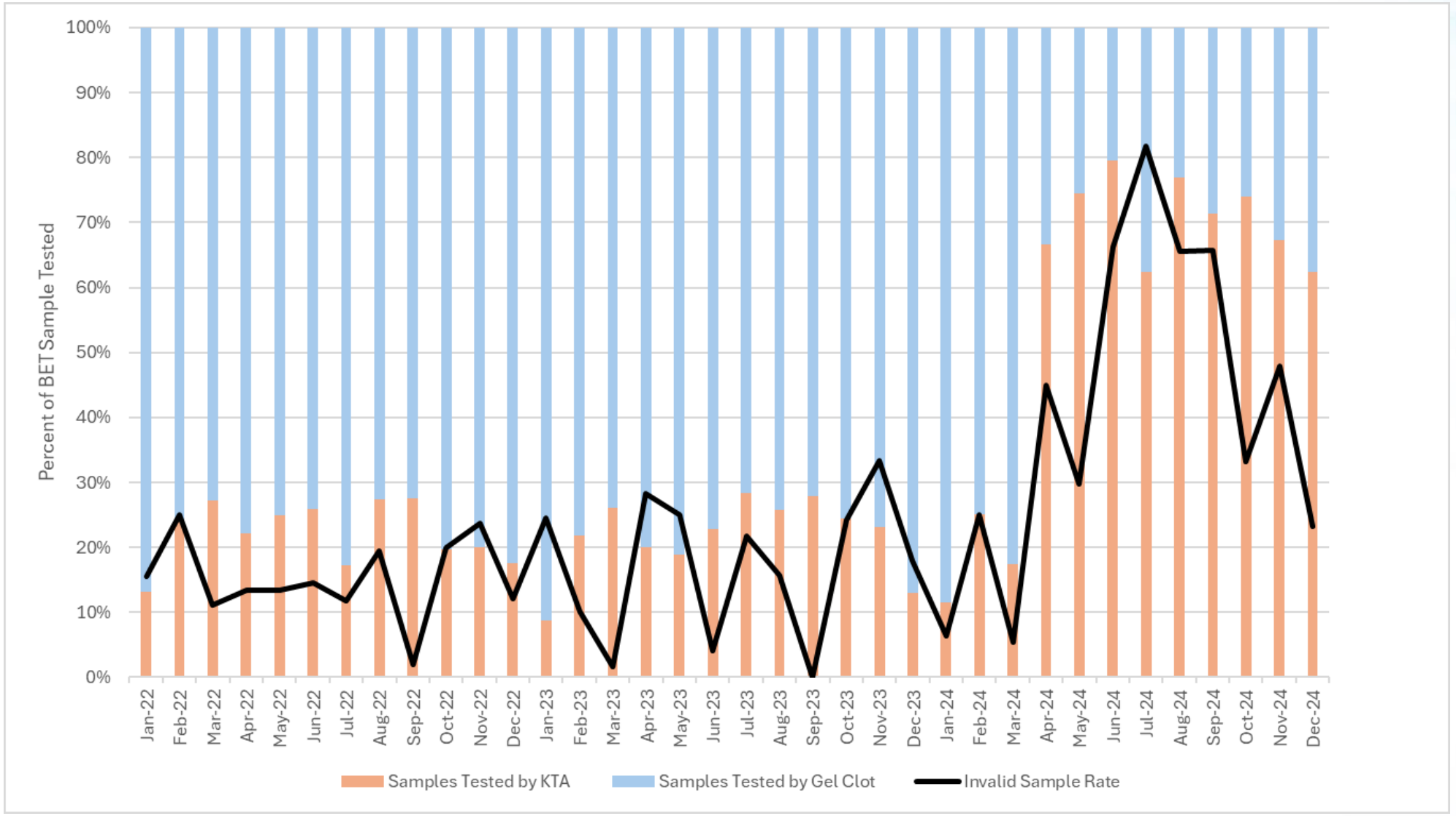
Case Study: Patterns - 2



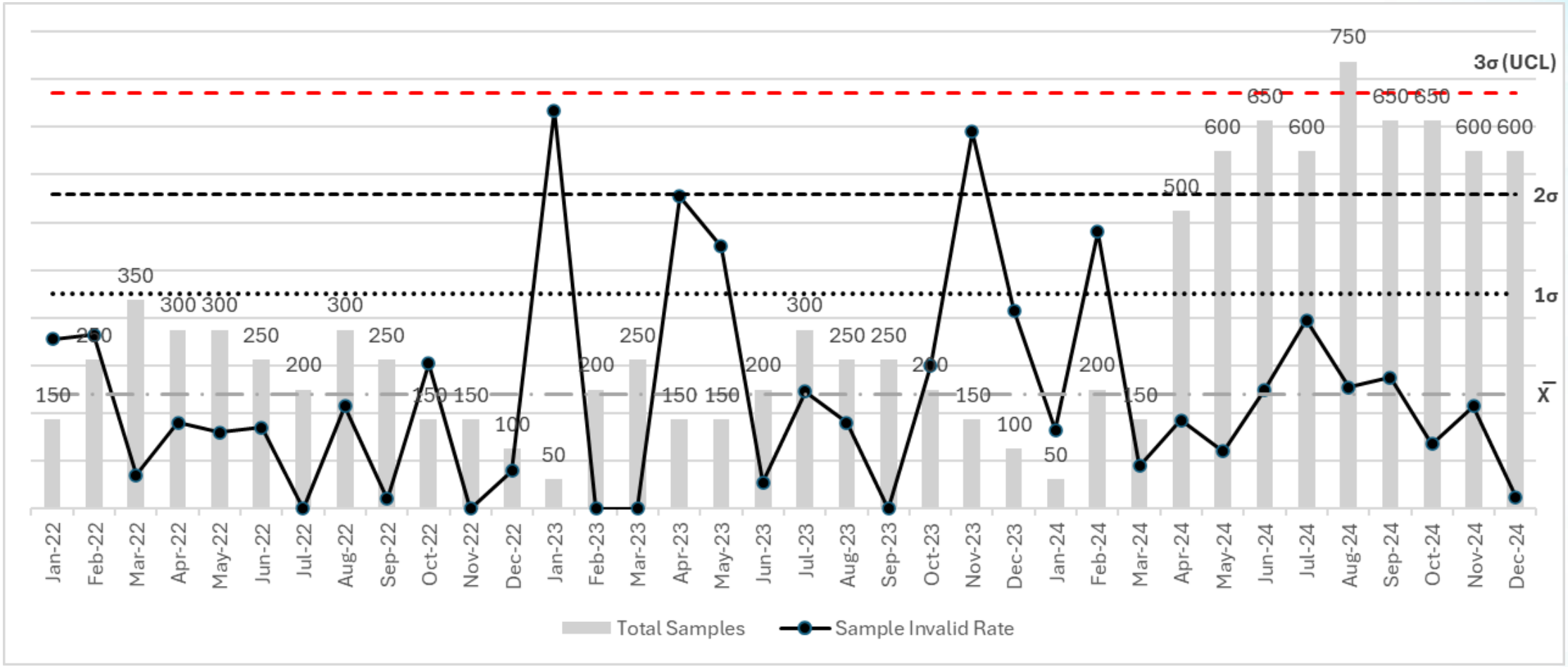
Case Study: Patterns - 3



Case Study: Gel Clot vs. Kinetic



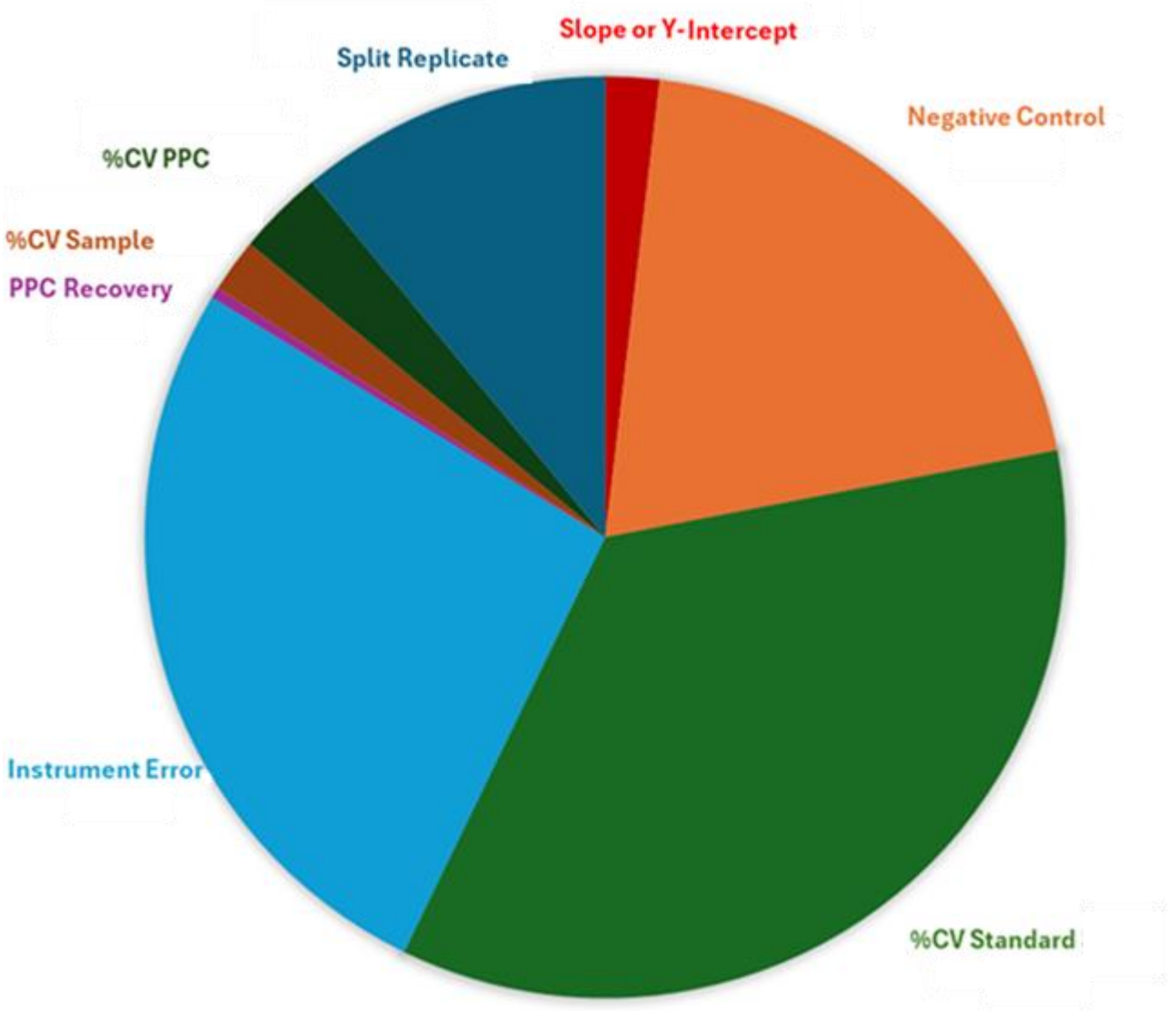
Case Study: Sample Size per Month



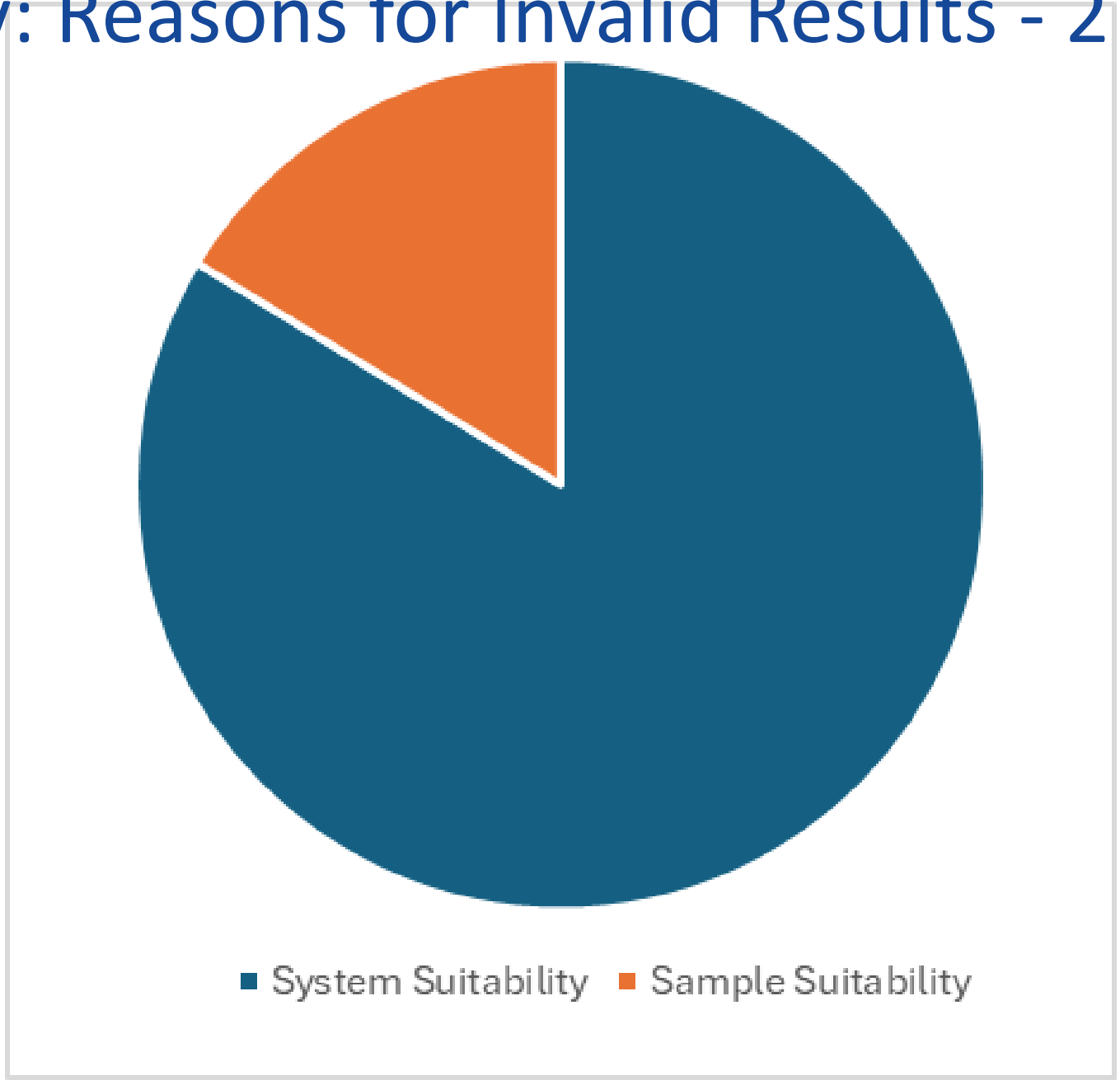
SPC Global Rules

1) **Standard formulas and trend frequency – Samples that are invalid and not invalid events**
2) **Invalid rate of each METHOD should be trended independently (Gel vs. kinetic are completely different)**
3) **Never compare two laboratories by invalid mean alone**
4) **Exceeding levels indicate POTENTIAL shifts in control**
5) **Importance of not falling behind on the data review**
6) **Consistent trend frequency across the network (when possible)**
7) **Provide appropriate training material and check-in with sites regularly**
8) **Don’t be afraid to use a level of subjectivity when trending…this is meant to be proactive**
9) **Empower employees to address the subjectivity**

Case Study: Reasons for Invalid Results - 1



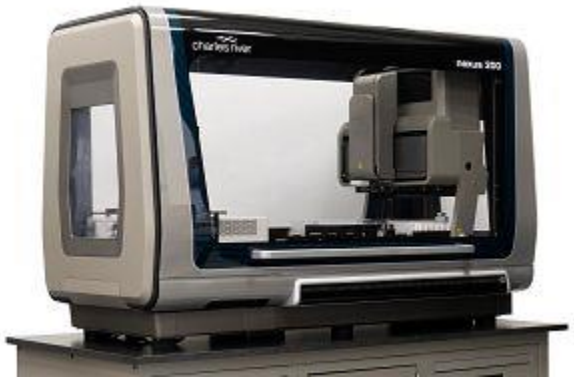
Case Study: Reasons for Invalid Results - 2



Future Method Improvements

1) Cartridge System

- Reduces analyst influence on test and single well anomalies
  - Robotics can eliminate the human influence on dilutions
- Potential system suitability invalid reduction
  - Reduces need to manually create standard curve
- Reduces impact of dispensing spike



2) Current Status

- India site has not implemented a cartridge system…yet
- Only a few sites in our network have implemented a cartridge system
- Our SMEs are pushing for all our sites to at least implement the cartridge system, and for high output sites to consider robotics

Project Conclusions

1) FDA Acceptance

- Site was able to overcome the OAI status
- Primary risk identified originally was the BET program and FDA was ok with the improvements made

2) Manufacturing Site

- Has become the leader in our manufacturing network in technical competency
- Consistently trend
- Proactive mitigation

3) Global Impact

- Improved BET training: pipetting and technical competence
- Identified a global solution to trend analysis

4) Future Considerations

- Cartridge systems
- Reduce pipetting precision differences through automation
- Continuously improve education materials

Special Thanks: Joe Wolinski, Jeff Dihel, and the Baxter Ahmedabad Team