

Trend Analysis for Long-Term Clinical Studies to Address Method Performance, Critical Reagents Bridging, and Stability Extension

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INTRODUCTION

Trend analysis provides a robust, data-driven framework for proactive monitoring and scientific oversight throughout the bioanalytical method lifecycle. Tracking performance patterns over time across analysts, instruments, reagent lots, and other critical variables enables the early detection of assay drift and accelerates root-cause identification during investigations. This systematic approach also strengthens critical reagent (CR) qualification, supports stability extensions, and informs the refinement of acceptance criteria for long-term clinical programs. Ultimately, implementing continuous trend monitoring strengthens assay robustness and provides a scalable foundation for operational decision-making and continuous process improvement.

METHOD

Table 1. Trend Analysis Methodology

Ligand Binding PK Assays	Immunogenicity Assays
<ul style="list-style-type: none"> Identification of patterns and/or shifts in data over time, typically when observed over 3 consecutive occasions. All accepted and rejected runs are included in the assessment, with the exception of analyst qualification data or results impacted by documented technical oversights. Data is plotted chronologically, by method. Analysts, instruments and critical reagents lots are noted and used for associations in trend review. 	<ul style="list-style-type: none"> Data trending via Levy-Jennings Chart Monitoring Controls: Negative Control (NC), Low and High Positive Control (LPC, HPC), signal-to-noise ratios (S/N), and % Inhibition and any additional control used for run acceptance (e.g., cell or assay buffer controls) Boundaries for trending of STDs: $\pm 3\sigma$ for upper and lower boundaries based on signal Boundaries for trending of QCs: %Bias $\pm 20\%$ based on concentration, or as applicable for the method

RESULTS

Identification of Additional Critical Reagent(s) During PK Clinical Bioanalytical Phase

Observations:

- 5/10 runs conducted over three consecutive days outside acceptance criteria.
- Runs used qualified CR and STDs/QC preparations for the PK method.
- Use of alternate lots of CR/preparations/splits – observation not resolved.

Investigation and Results:

- A trend analysis was performed using data from all regressed runs.
- A specific assay buffer lot change was noted for runs 62-73 performed within 5 days, which was associated with a consistent reduction in LLOQ signal (from ≥ 500 RLU to ≤ 180 RLU), including five rejected runs and accepted runs (Table 2, Runs 62-73) as shown in Figure 1. ULOQ showed no alterations.
- The suboptimal performance was attributed to the buffer lot in-use compared with the previous lot.
- A change in assay buffer lot recovered the LLOQ signals to the expected levels (≥ 500 RLU approximately) from Run 74 onwards (Table 2).

Table 2. Representative RFU Signals Values Presented for Assay LLOQ and ULOQ

RUN ID	LLOQ STD	ULOQ STD
59	628.500000	607390.500000
60	662.500000	664157.500000
61	614.500000	616999.500000
62	178.500000	512683.500000
63	196.000000	516799.500000
64	159.000000	643052.500000
65	168.000000	663414.000000
66	147.000000	524898.000000
67	144.500000	525576.000000
68	163.500000	611334.000000
69	184.500000	635037.500000
70	147.000000	479830.000000
71	141.500000	471203.500000
72	176.500000	563364.500000
73	153.500000	492971.000000
74	659.500000	631105.000000
75	646.500000	642379.500000
76	802.500000	670948.500000
77	801.000000	667419.500000

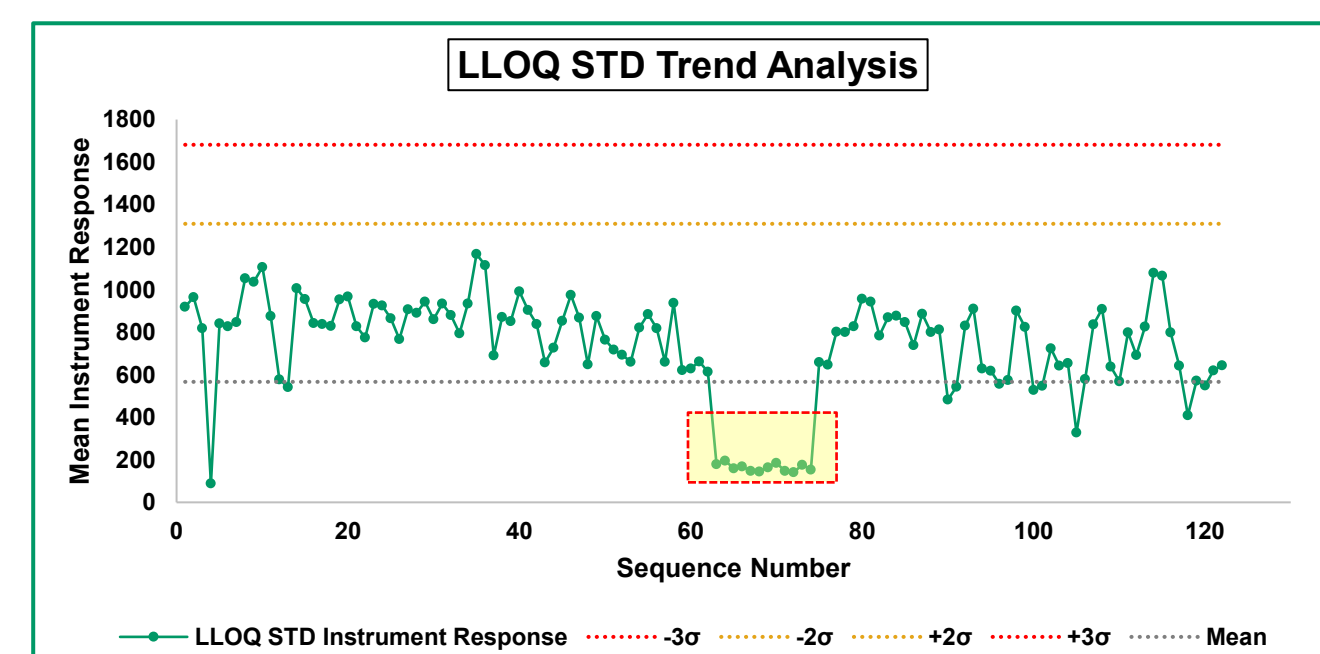


Figure 1. Graphical Presentation of LLOQ Signals

Conclusion:

- Assay buffer was designated as a critical reagent for this PK method to ensure it is monitored and qualified each time a new lot is introduced.

Extension of Critical Reagent(s) Stability

Observations:

- Since minor lot-to-lot variability in critical reagent performance can be expected which may contribute to gradual drift in assay over time, which can be avoided by extending the re-test dates for the critical reagent lot.
- Appropriate monitoring strategies should be implemented to assess stability and establish a new retest date for such critical reagent(s).

Investigation and Results:

- Certain critical capture reagents such as probes, may be supplied without defined retest or expiration dates. Data from >100 runs performed for a long-term clinical study were monitored to assess CR stability and extension of re-test dates.
- A consistency in assay LLOQ (STD1) and ULOQ (STD10) signals within $\pm 2\sigma$ were observed. LQC/HQC concentration scatterplot within $\pm 20\%$ bias (Figures 2 and 3) were also observed, representing over 94% of all runs, suggest a consistent performance of this CR beyond originally assigned retest date of six months.

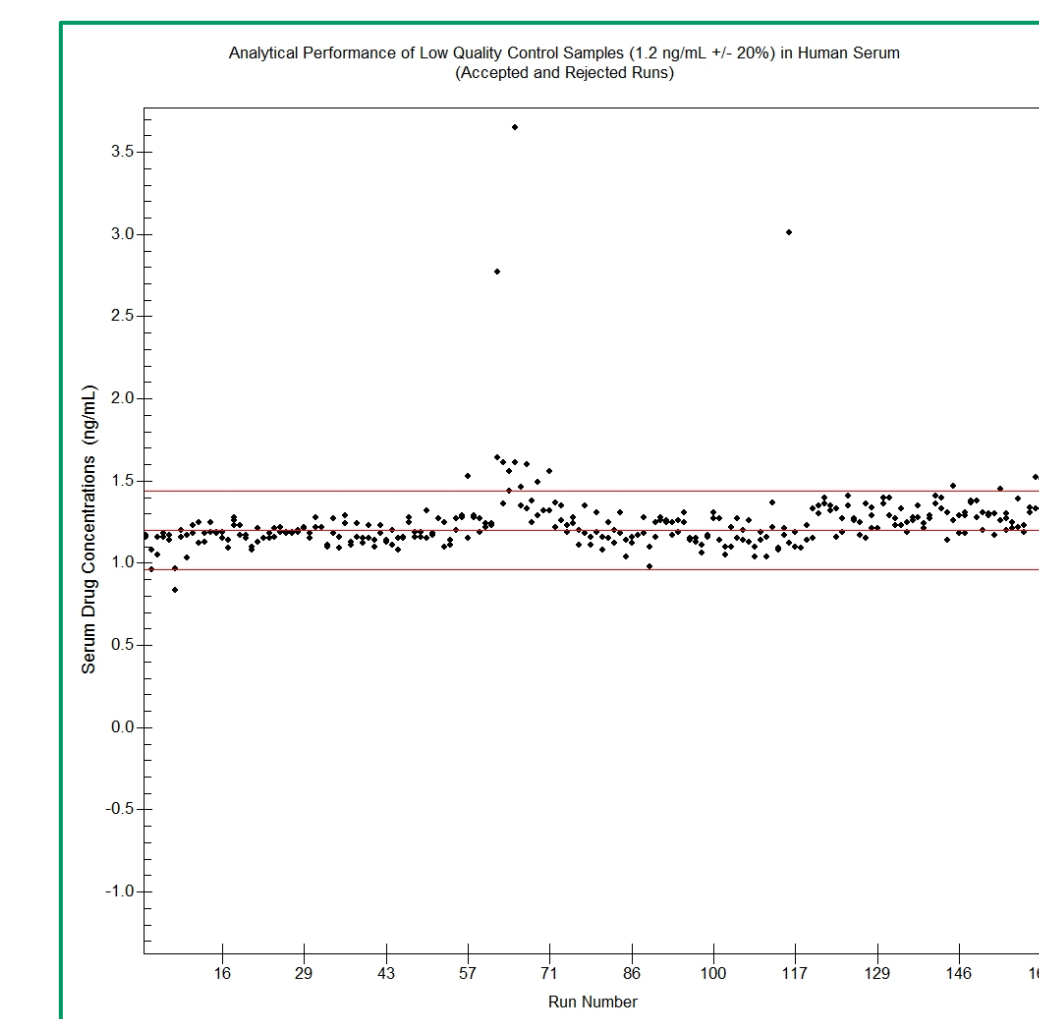


Figure 2. Scatter Plot of LQC Samples

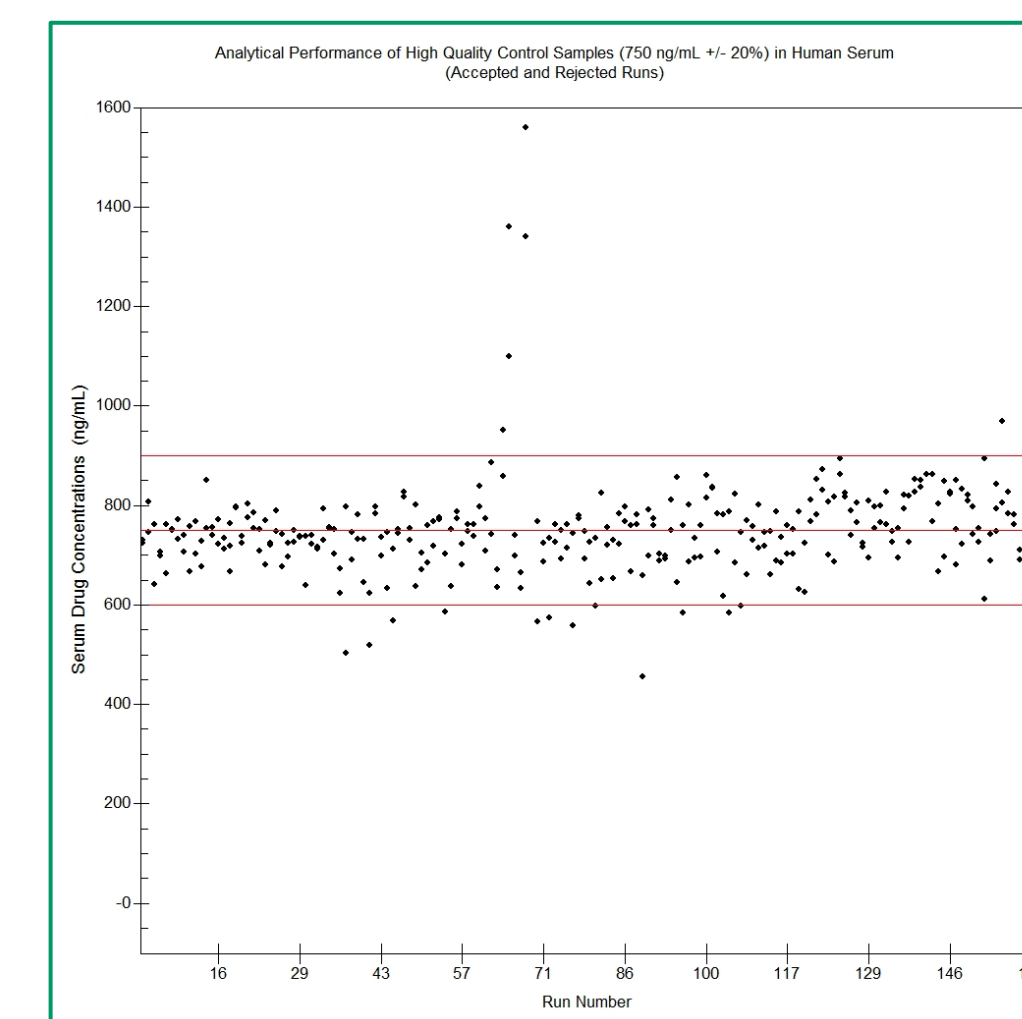


Figure 3. Scatter Plot of HQC Samples

Conclusion:

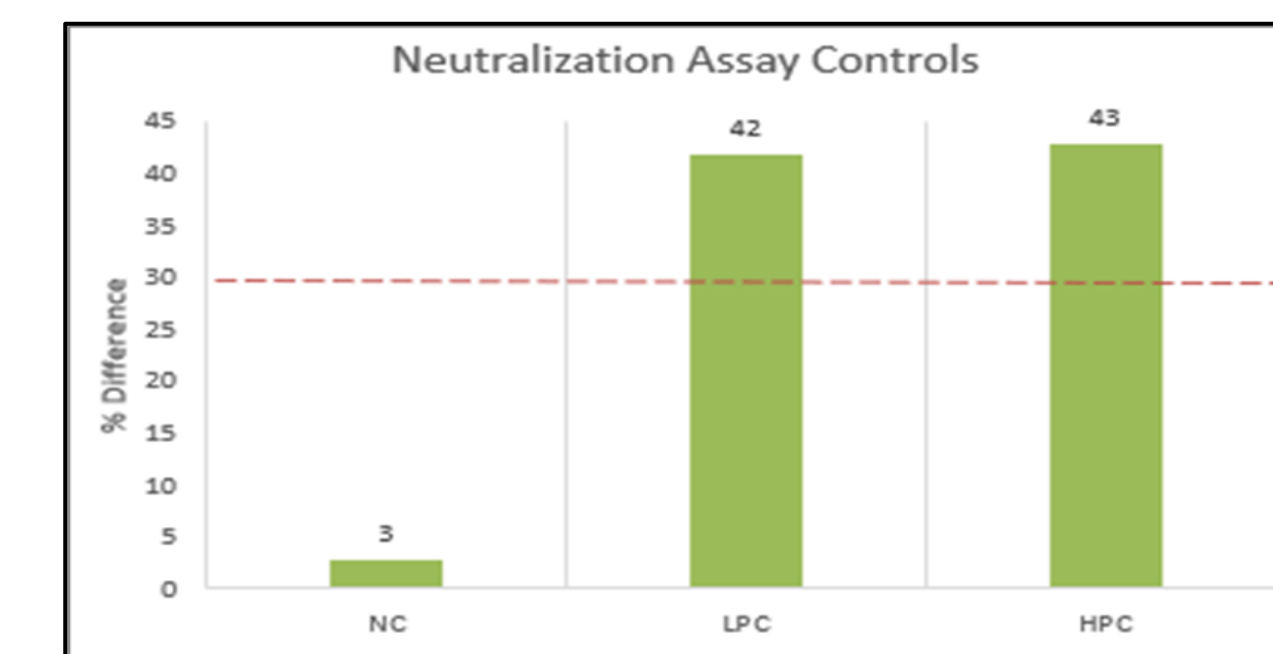
- This approach demonstrates how trend analysis can be effectively leveraged to ensure ongoing reagent suitability while supporting long-term clinical study continuity.
- Using this approach, the retest dates for these probes were extended by 18-24 months (reagent volumes permitting) beyond the supplier-assigned retest date.

Critical Reagent Qualification During a Neutralizing Anti-Drug Antibody(Nab) Competitive Ligand Binding (CLB) Assay

Observations (Figure 4):

- During a head-to-head critical reagent qualification of a new lot of Pierce streptavidin-coated magnetic beads (performed in one physical plate), % difference > 30 was observed for the LPC and HPC% inhibition between the qualified lot and a new lot to be qualified
- The NC% Inhibition between the qualified lot and a new lot to be qualified was within 30% difference

Figure 4. Percent Difference Between Old and New Critical Reagent During Head-to-Head Qualification



Investigation and Results (Figures 5 and 6):

- Trend analysis using historical data for NC, LPC, and HPC controls trended (data showed for LPC and HPC only): No trend identified, and the new CR is performing as per past assay performance.
- Calculation of % difference between four replicates of the new lot of CR and the mean of historical data using the qualified lot: NC, LPC, and HPC are within 30% difference

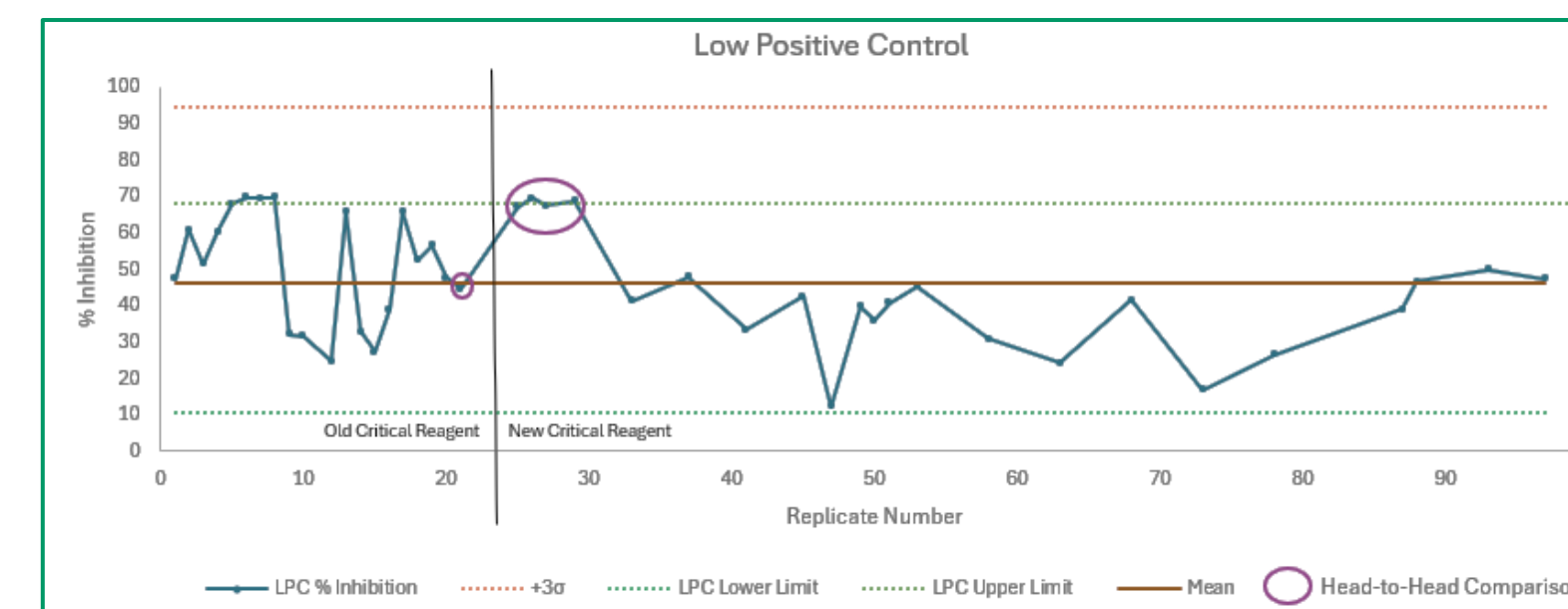


Figure 5. Levy-Jennings Chart for LPC Trending During a Nab CLB Method

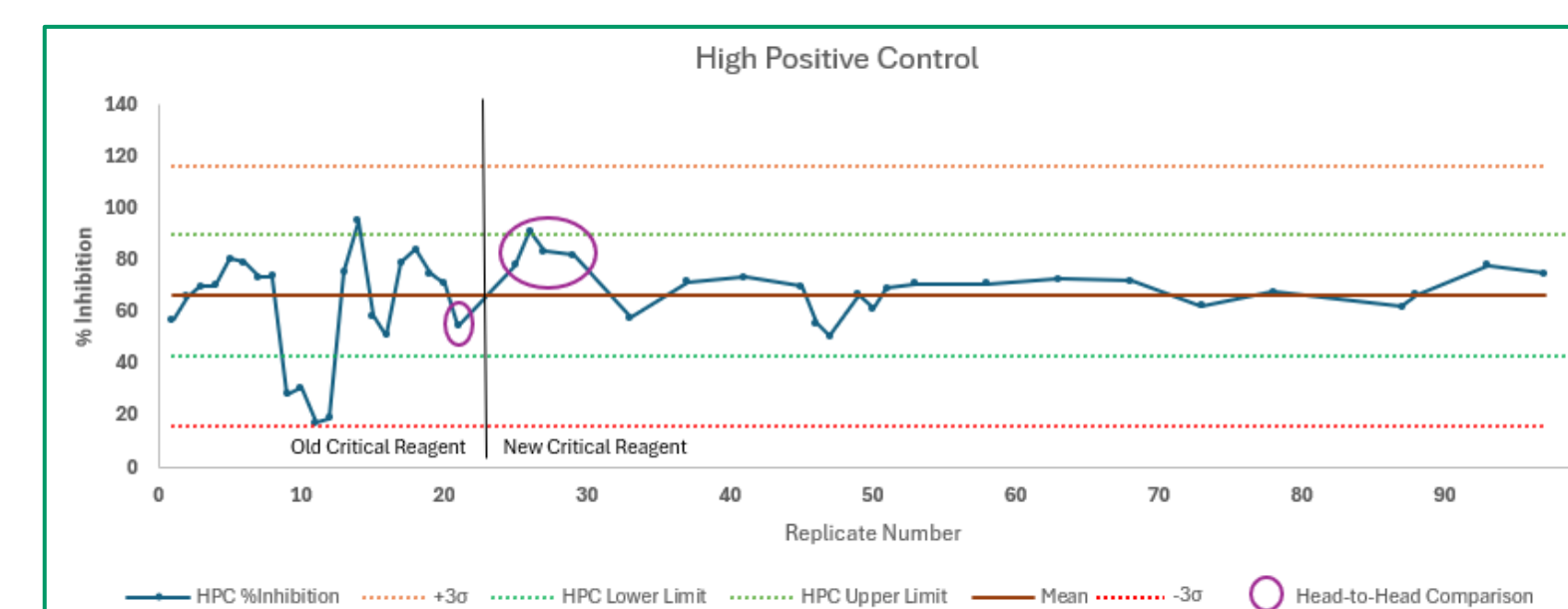


Figure 6. Levy-Jennings Chart for HPC Trending During a Nab CLB Method

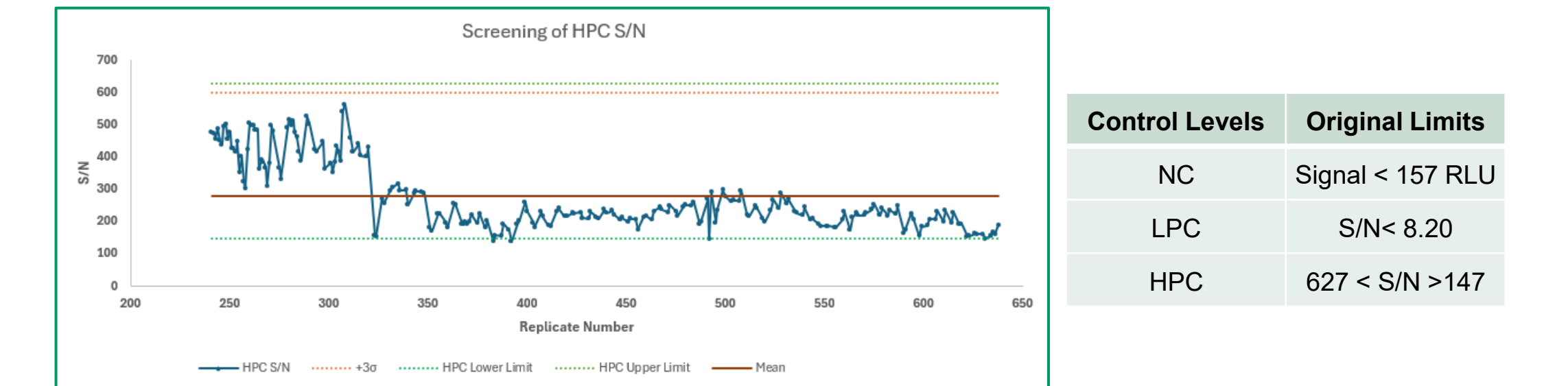
Conclusion:

- Head-to-head comparison is a rapid and isolated way to qualify a new critical reagent and does not account for normal method variability over time.
- Trend analysis and historical data tabulation successfully supported the qualification of the new critical reagent
- Trend analysis allows monitoring of the new critical reagent over time to ensure its performance is maintained and comparable to the previously qualified lot.
- Continued use of the new streptavidin-coated magnetic beads further demonstrates its comparability to the previously qualified lot.

Monitoring and Adjustment of Immunogenicity Control Suitability Acceptance Limits

- Trend analysis is a valuable tool to visualize shifts in control levels over time, as demonstrated by the shift in HPC signal noted during method validation (Figure 7), allowing for prompt assessment and resolution.
- All validation data were tabulated for system suitability limit determination and successfully supported over 200 sample analysis runs across three long-term clinical studies.

Figure 7. Trend Analysis of High Positive Control at Final Suitability Level During Method Validation



Observations:

- During sample analysis, following 230 runs, three consecutive runs failed to meet acceptance criteria due to the HPC S/N being 9% lower than the established lower limit.
- Trend analysis demonstrated that the analyst, days, instruments, and critical reagents lots were not contributing to the run failures. All other assay controls (NC, LPC, and inhibition NC, LPC, and HPC) were performing appropriately.
- 84 data points at the final suitability HPC level were used to establish the suitability limits during method validation (Mean HPC S/N +/- t0.01, df x SD, where t0.01, df corresponds to a 1% failure rate, one-tailed).

Investigation and Results:

- Trend analysis showed that the HPC S/N from the failed runs was not indicative of a trend, and similar to the previous HPC S/N ratios were observed.
- HPC performance was always within the $\pm 3\sigma$ S/N boundaries (-3σ limit not shown as below 0).
- A hypothesis was stipulated that the validated system suitability limits for HPC were not representative of the performance of the assay over time.
- HPC system suitability limits were recalculated using all accepted runs, excluding the last three rejected runs (Figure 8), resulting in the inclusion of 485 data points for the HPC limits.
- Re-calculated limits resulted in the acceptance of the previously three rejected runs.
- Trending of the method following limit adjustment showed HPC is performing as per historical data observed.

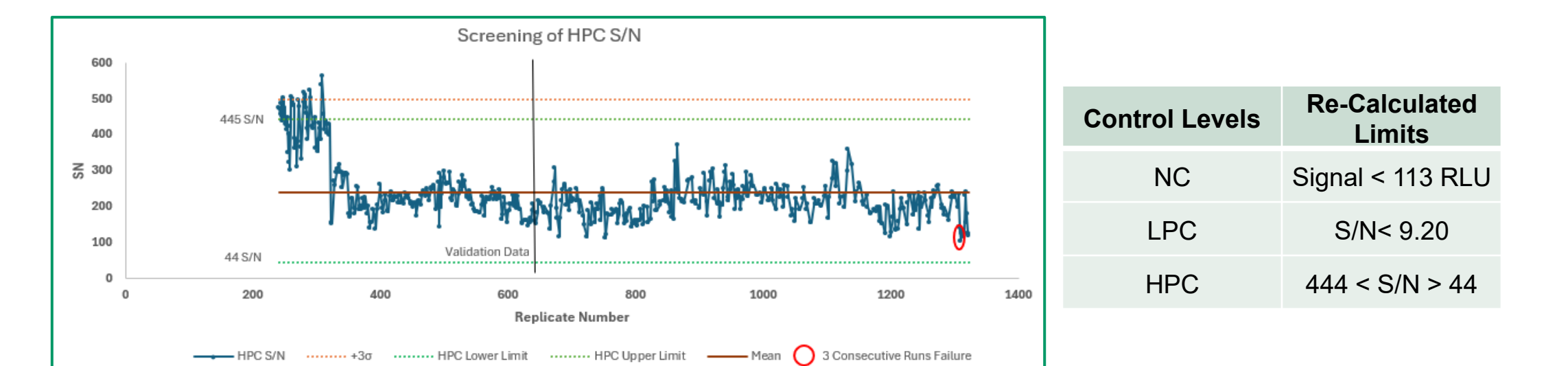


Figure 8. Trend Analysis of High Positive Control S/N Following Adjustment of Limits

Conclusion:

- The recalculated system suitability limits more accurately capture the true performance of the HPC over time. This highlights the importance of data monitoring using trend analysis control signals as well as the value of periodically re-adjusting limits based on the increasing number of data points generated throughout the assay's life cycle.

CONCLUSION

In long-term studies, minor lot-to-lot variability in critical reagents and/or preparations can contribute to gradual assay drift over time. Trend analysis enables continuous monitoring of assay performance, facilitates early detection of subtle signal changes, and helps ensure that immunogenicity acceptance limits, often established from limited validation data points, remain representative and reliable throughout the study/program duration.