Research Article

Triple Point Pooled Sera (TriPPS) QC for Laboratory Analyte Error Detection: A Machine Learning based Quality Control in Laboratory

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Article Info

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Keywords

Serum, Machine Learning, Quality Control, Medical Errors/ prevention & control, Clinical Laboratory Techniques

Abstract

Background: Reliable internal quality control (IQC) is vital for ensuring analytical accuracy in clinical laboratories. Conventional rule-based QC systems, such as Westgard and Levey–Jennings, often exhibit retrospective detection and limited sensitivity to small but clinically meaningful shifts. This study introduces the Triple-Point Pooled Sera (TriPPS) Quality Control system, a novel, machine-learning-based framework integrating in-house pooled sera with adaptive algorithms for enhanced error detection.

Methods: Residual patient sera were pooled to create stable, matrix-relevant IQC material for 60 consecutive analytical days. Sodium and potassium were used as representative analytes. Three complementary machine learning models were applied: k-Nearest Neighbour (k-NN) for trend detection, Isolation Forest (IF) for random error identification, and Gaussian Process Regression (GPR) for systematic bias modeling. Controlled ±1% daily biases and stochastic random errors were introduced to simulate analytical drift. Detection lag, sensitivity, and anomaly classification were evaluated.

Results: The k-NN algorithm effectively identified trend errors within 0–2 days of bias onset, while IF accurately detected random fluctuations with minimal false positives. GPR modeled nonlinear systematic drift with high fidelity, capturing bias progression that is overlooked by linear methods. The integration of pooled sera enhanced the system's stability, reproducibility, and cost efficiency across all error types.

Conclusion: The TriPPS system demonstrates a scalable, data-driven approach to laboratory quality control by combining pooled sera with machine learning algorithms. This framework enhances analytical vigilance, facilitates proactive error identification, and provides a practical, resource-efficient solution for real-time QC monitoring in clinical chemistry laboratories.

Introduction

Precise and accurate biochemical testing is crucial for patient management; as clinical decisions often rely on laboratory-generated data. Therefore, robust internal quality control (IQC) systems are crucial for ensuring analytical accuracy, minimizing reporting errors, and safeguarding patient safety [1, 2]. IQC consistently assesses intra-laboratory variability to ascertain whether analytical variations remain below acceptable thresholds and detect possible deviations before they affect outcomes. Despite the prevalent use of commercial control materials, their elevated cost, lot-to-lot variability, and sporadic unavailability continue to pose significant obstacles, especially for laboratories in low- and middle-income regions [3]. Conversely, in-house pooled sera have emerged as a practical and dependable alternative, providing a matrix equivalent to native patient samples while significantly reducing costs and logistical reliance [1, 4, 5]. Pooled sera have demonstrated significant efficacy in detecting both random and systematic analytical mistakes, therefore maintaining analytical consistency over prolonged monitoring intervals [4].

Considering their evident potential, pooled-sera-based IQC programs continue to focus significantly on conventional statistical process control methodologies, like Levey-Jennings plots and Westgard multi-rule criteria [6, 7]. Although these approaches have been fundamental to clinical laboratory quality control for decades, they are fundamentally retroactive, identifying out-of-control occurrences only after a substantial deviation has transpired. Analytical alterations may remain unnoticed for several hours or even days prior to intervention [8, 9]. Furthermore, these traditional rule-based systems have restricted sensitivity to minor but clinically significant variations, resulting in misleading confidence in analytical stability [10–13]. The growing intricacy of automated laboratory systems, combined with increased sample throughput, has exacerbated manual validation procedures, thereby heightening the likelihood that minor or transient errors go undetected [10]. These constraints underscore the urgent necessity for more flexible, immediate, and data-informed methodologies in laboratory quality assurance.

Recent advances in machine learning (ML) present a significant opportunity to address these limitations. Machine learning algorithms possess the capability to independently learn from historical data patterns, identify intricate, non-linear relationships, and detect anomalies with significantly greater sensitivity compared to conventional quality control rules [10, 12, 14, 15]. In the context of laboratory analytics, machine learning can enhance the early detection of random noise and systematic drift, thereby connecting statistical process control with predictive maintenance [10]. Incorporating machine learning into standard quality control procedures has the potential to enhance the reliability of analytical outcomes and establish ongoing, intelligent monitoring systems in clinical laboratories.

This study presents and assesses the Triple-Point Pooled Sera (TriPPS) Quality Control system, an innovative machine-learning-based QC model designed for the detection of laboratory analyte errors. The TriPPS approach integrates pooled sera as a cost-effective, matrix-relevant control material alongside a suite of machine learning algorithms, such as k-Nearest Neighbour (k-NN), Isolation Forest (IF), and Gaussian Process Regression (GPR), to identify and categorize analytical deviations. The term “Triple-Point” in TriPPS reflects the integration of three complementary analytical layers within a unified QC framework: (1) matrix-relevant pooled sera as the material foundation, (2) multi-domain error detection encompassing trend, random, and systematic deviations, and (3) three distinct machine learning algorithms optimized for each error category. This tripartite architecture enables simultaneous surveillance across multiple dimensions of analytical instability. This study seeks to create a comprehensive, scalable QC framework that simultaneously tackles trend, random, and systematic errors, thereby enhancing analytical vigilance and resilience in clinical chemistry laboratories.

Methodology

The study was conducted in the Department of Biochemistry at All India Institute of Medical Sciences. As only discarded samples were used, the Institutional Ethical Committee waived the need for ethical clearance.

Preparation of Pooled Sera

The estimated requirement of serum for each analyte across the study duration was approximately 100 µL per run; thus, for a 60-day evaluation period, a total of 6 mL of pooled sera was required. The pooled sera were prepared on a single day (Day 0) using residual and discarded patient serum samples collected from the clinical biochemistry laboratory. Based on patient reports, only samples falling within or close to the reference intervals were selected to ensure representative analytical performance. Samples exhibiting visible haemolysis, lipemia, icterus, or insufficient volume were excluded to preserve analytical integrity.

Although individual samples were not screened for infectious pathogens such as HIV, Hepatitis B, or Hepatitis C, owing to the retrospective and pooled nature of the work, strict adherence to universal biosafety precautions was maintained. Personnel used gloves, laboratory coats, and face shields, and followed institutional protocols for hand hygiene and surface disinfection. The collected sera were thoroughly homogenized to ensure compositional uniformity, then aliquoted into 60 sterile 250 µL microcentrifuge tubes using calibrated micropipettes. All aliquots were immediately frozen at –5 °C in a dedicated container to maintain analyte stability throughout the 60-day quality-control (QC) period. For each analytical day, one aliquot was thawed and analysed in parallel with commercial QC material, serving as an internal stability comparator.

Analytical Workflow and Data Acquisition

Each pooled-sera aliquot was assayed using the routine biochemistry analyzer under identical operational conditions to minimize instrument-related variation. Daily analyses were performed twice, once in the morning and once in the evening , to simulate two independent QC runs and to assess possible temporal drift. Electrolytes, specifically sodium and potassium, were chosen as model parameters due to their analytical robustness and relevance in monitoring instrument performance. Raw measurement data were recorded for each run and compiled into a longitudinal dataset spanning 60 consecutive days.

To evaluate the models' detection capability, systematic and random errors were artificially induced. A controlled linear bias of ±1% per day was introduced into the dataset to simulate analytical drift (trend error). In contrast, stochastic random errors were injected at predefined intervals to represent transient measurement noise. Each day's run was labeled as Normal, Amber (with a deviation of more than 75% from the baseline mean), or Red (with a deviation of more than 90% from the baseline mean). These percentage thresholds were selected to represent graded deviation severity relative to the induced bias magnitude, allowing discrimination between moderate and high analytical instability. The cut-offs were intentionally set conservatively to enhance sensitivity during proof-of-concept validation rather than to replicate specific regulatory decision limits.

Machine-Learning Framework

Three complementary machine-learning algorithms were employed to identify analytical anomalies: k-Nearest Neighbour (k-NN) for trend detection, Isolation Forest (IF) for random error detection, and Gaussian Process Regression (GPR) for systematic bias modelling.

k-Nearest Neighbour (k-NN) for Trend Detection

The k-NN model was used to capture gradual changes in the pooled-sera measurements over time. A sliding-window length of 3 days and a neighbourhood size of 1 were adopted after sensitivity testing for optimal performance. The algorithm computed local similarity among consecutive runs based on Euclidean distance, flagging deviations that exceeded predefined drift thresholds. Detection output was expressed as the percentage of Red and Amber days, as well as the lag between the onset of bias and the first detection event.

Isolation Forest (IF) for Random-Error Detection

The IF algorithm, an ensemble-based anomaly detection approach, isolates data points through recursive random partitioning of the feature space. Each measurement's anomaly score was determined by its average path length across a forest of randomly constructed trees (n = 100). Points with shorter path lengths corresponded to higher likelihoods of anomalies. The model was trained on bias-free baseline data and subsequently applied to datasets containing injected random noise to quantify sensitivity and false-flag rate.

Gaussian Process Regression (GPR) for Systematic-Bias Analysis

GPR was employed to model structured, non-linear deviations resulting from gradual bias induction. Using a radial-basis (Gaussian) kernel, the regression function estimated posterior mean predictions and associated uncertainty intervals for each daily observation. Divergence between observed and predicted values was interpreted as evidence of systematic drift. The flexibility of GPR enabled comparison of linear versus non-linear bias progression across both electrolytes.

Evaluation Metrics and Visualization

For each algorithm, performance was assessed using the proportion of flagged Red and Amber days, the lag (in days) to first detection, and visual inspection of anomaly or residual plots. Model outputs were summarized numerically for sodium and potassium across morning and evening sessions. All analyses were performed using Python (version 3.12) with standard scientific libraries , NumPy, pandas, scikit-learn, and matplotlib. The reproducible codebase was validated using repeated subsampling to ensure stability of detection patterns.

Results

This study consisted of a continuous 60-day assessment of pooled serum samples for electrolytes, with a focus on sodium and potassium as indicative analytes of analytical stability. We examined the morning and evening batches separately to determine if there was any variation in measurement drift throughout the day. The k-Nearest Neighbor (k-NN) model was initially utilized to identify linear trends induced by a controlled ±1% bias per day. Categorizing variations into Amber (>75%) and Red (>90%) zones allowed it the potential to measure the model's sensitivity and the period it takes to calculate flags.

The k-NN-based trend detection framework showed the same level of accuracy for both electrolytes. A +1% per-day trend for sodium led to 80% Red-day identification in morning runs and 93.3% in evening runs. A -1% trend, on the other hand, led to 73.3% and 100% detection, respectively. Potassium exhibited a comparable trend, achieving full (100%) detection of negative drift during evening sessions, with an average delay of 0–2 days prior to the initial alert. These results demonstrate that the model rapidly adapts to new analytical biases. This has been summarized in Table 1.

Table 1: Analysis of Trend Detection by k-NN method.

Electrolyte	Run	Error Type	No of Red Days	Red Days (%)	No of Amber Days	Amber Days (%)	First Day of Error Detected	Lagged Days
Sodium	Morning	Trend +1%/ day	12	80	2	13.3	47	1
	Morning	Trend -1%/ day	11	73.3	2	13.3	48	2
	Evening	Trend +1%/ day	14	93.3	1	6.7	46	0
	Evening	Trend -1%/ day	15	100	0	0	46	0
Potassium	Morning	Trend +1%/ day	13	86.7	1	6.7	47	1
	Morning	Trend -1%/ day	13	86.7	2	13.3	46	0
	Evening	Trend +1%/ day	12	80	2	13.3	47	1
	Evening	Trend -1%/ day	15	100	0	0	46	0

The IF algorithm was subsequently utilized to detect random error occurrences within aggregated sera datasets. IF effectively separated sporadic anomalies from background analytical variation by successively segmenting the feature set and defining points with short mean path lengths. In potassium runs, random error induction caused sharp, isolated spikes in anomaly scores that matched the injected noise exactly. In sodium runs, similar short-term changes were found without over-flagging. These observations validate the adaptability of IF in identifying stochastic instability. Figure 1 and 2 illustrate this.

Figure 1: Detection of Random Error in Potassium Pooled Sera Measurement by IF.

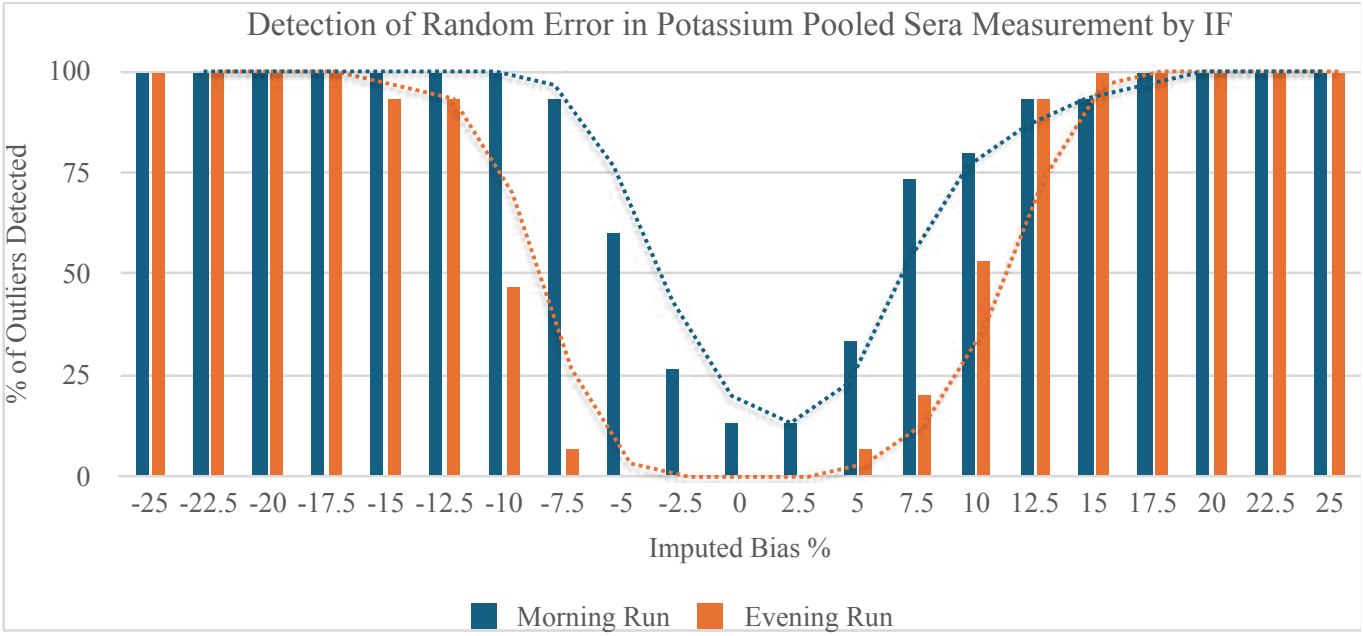
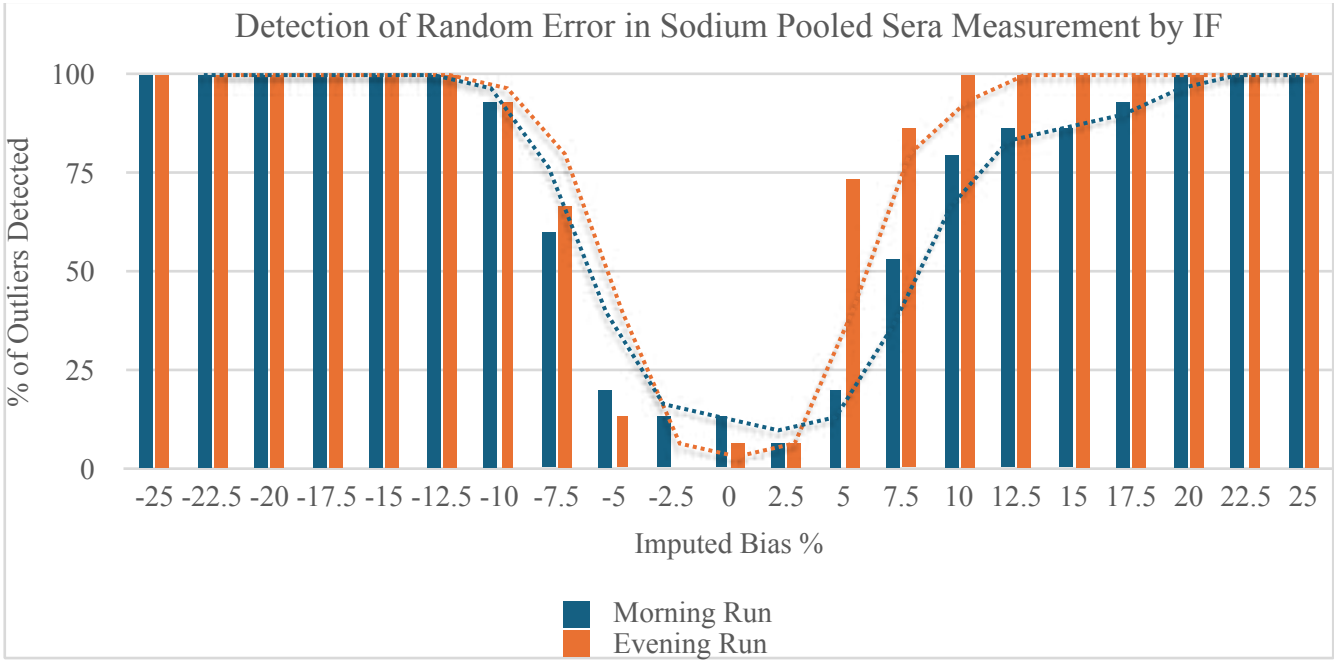


Figure 2: Detection of Random Error in Sodium Pooled Sera Measurement by IF.



Next, Gaussian Process Regression (GPR) was used to examine systematic bias in datasets that had been purposely imputed. In potassium, GPR recorded the progressive deviation of estimated versus measured values as the bias magnitude increased, showing uniform, non-linear shift dynamics. Sodium acted similarly, and Gaussian kernels accurately mapped the residual expansion over time. These results highlight GPR's capacity to model structured deviations that evade detection by linear approaches alone. Figure 3 and 4 depict this.

Figure 3: Systemic Error Detection in Biased Imputation of Potassium by GPR.

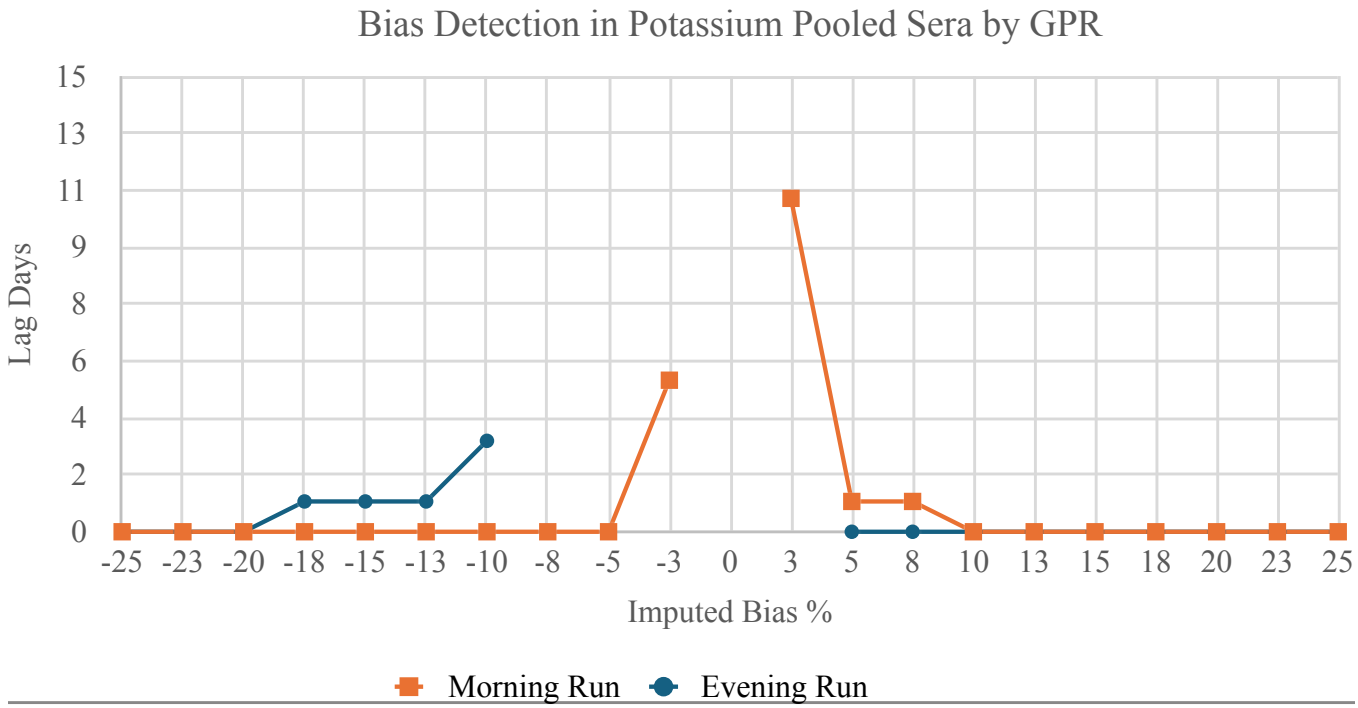
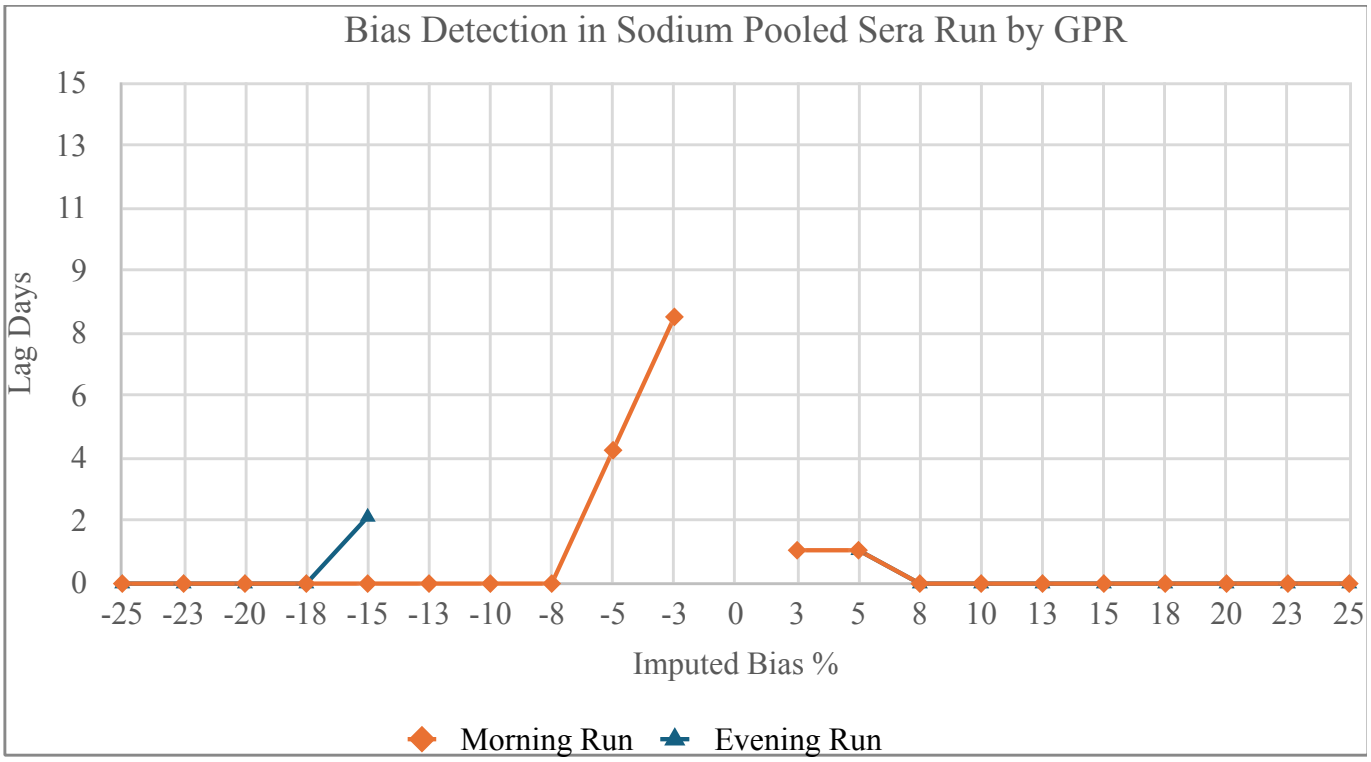
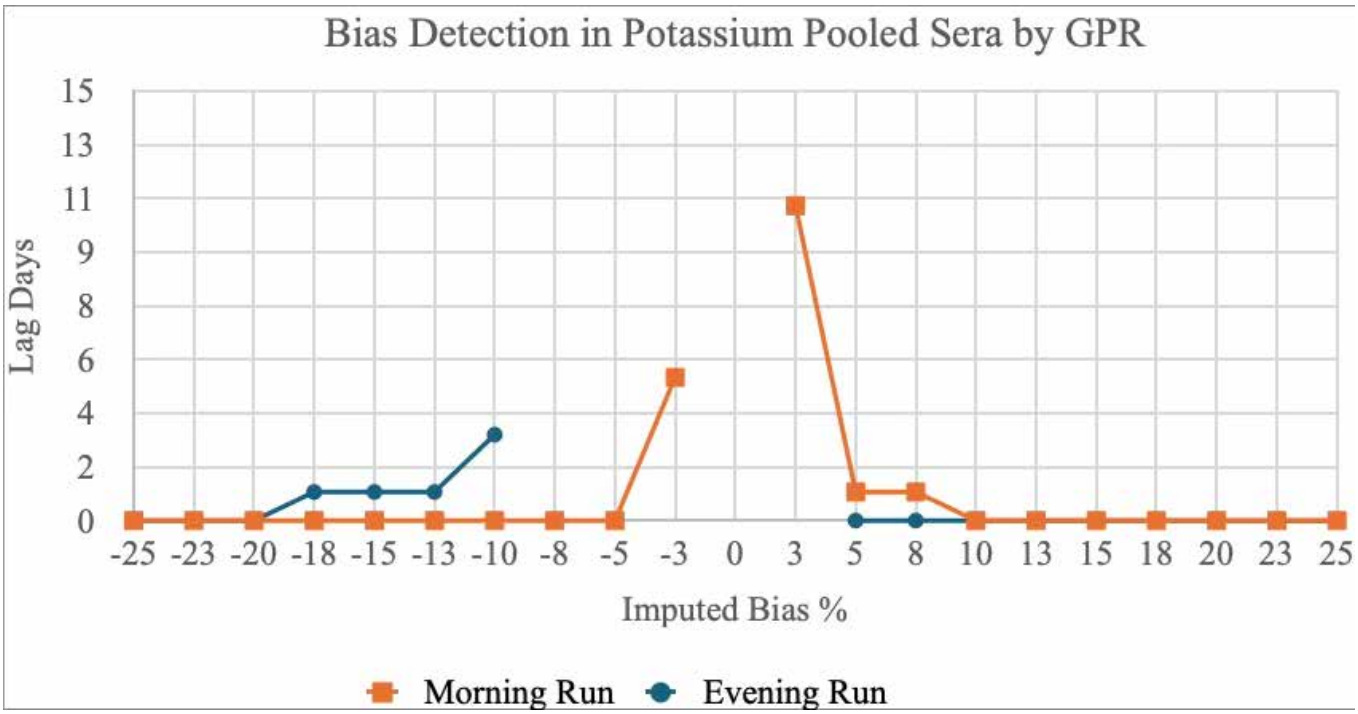


Figure 4: Systemic Error Detection in Biased Imputation of Sodium by GPR.





Discussion

This study presents and assesses the Triple-Point Pooled Sera (TriPPS) Quality Control system, a contemporary machine learning framework designed to enhance the detection of laboratory analyte errors. Through the integration of pooled sera, a cost-effective, matrix-relevant internal control, alongside a suite of complementary algorithms, the TriPPS system creates a multi-layered framework for thorough analytical monitoring. In this study, the k-Nearest Neighbour (k-NN) method was utilized for trend detection, the Isolation Forest was applied for identifying random errors, and the Gaussian Process Regression (GPR) was employed for modeling systematic bias. The system exhibited strong sensitivity in detecting anomalies across various error types by intentionally introducing linear trends and stochastic random errors. The collective findings indicate that the TriPPS model has the potential to enhance analytical vigilance and resilience in clinical chemistry laboratories, especially in environments where resource constraints limit the regular use of commercial control materials.

The enhanced features of machine-learning-driven quality control systems effectively tackle numerous persistent challenges associated with traditional IQC approaches. Traditional rule-based systems, such as Westgard rules or Levey–Jennings charts, depend on retrospective detection and exhibit limited sensitivity to minor but clinically significant deviations, resulting in a delay in identifying analytical instability [10]. In high-throughput automated laboratories, the reliance on manual validation exacerbates this issue, heightening the likelihood that minor drifts or temporary errors remain undetected [10]. Machine learning facilitates data-driven, real-time surveillance by employing adaptive modeling techniques [16, 17]. Through the analysis of historical data and the recognition of complex, non-linear relationships, machine learning-driven quality control frameworks are capable of identifying analytical drift or bias with enhanced precision and efficiency [18]. This capability facilitates the prompt identification of errors associated with calibration drift as well as pre-analytical and analytical disturbances, including sample contamination from intravenous fluids or anticoagulants, delays in analysis, or incorrect blood sample handling. Similar results have been observed in investigations assessing Machine Learning Internal Quality Control (MLiQC) systems, which have shown enhanced bias detection and early warning sensitivity compared to patient-based real-time QC approaches [12]. The convergence of these findings highlights the significant potential of machine learning in transforming quality control approaches from a reactive stance to a proactive monitoring system.

The integration of triple-point pooled sera into the TriPPS framework significantly enhances its practical applicability and methodological strength. Pooled sera, obtained from leftover patient samples, provide a cost-effective and compositionally genuine substitute for commercial controls, addressing challenges associated with cost, availability, and variability between lots [4, 5]. Their biological resemblance to patient matrices guarantees that the analytical performance metrics derived from pooled sera more precisely represent actual clinical scenarios. Previous investigations have demonstrated that pooled sera are effective in detecting both random and systematic analytical errors over extended periods [12, 19]. The TriPPS model enhances this foundation by integrating the stability of pooled sera with algorithmic intelligence, resulting in a cohesive system that can concurrently identify both stochastic and directional errors. This dual-layered integration connects material-based stability assurance with computational vigilance.

The application of ML in this scenario is designed not to replace traditional IQC methods, but to enhance and update them. Integrating statistical process control with predictive maintenance allows for the early identification of emerging noise or drift, thereby evolving quality control into a system of continuous learning [20, 21]. These systems can independently identify unusual runs, anticipate calibration issues, and prioritize samples for manual examination, thus enhancing analytical precision and operational effectiveness [18]. The enhancement of conventional quality control methods leads to fewer undetected errors, quicker corrective measures, and heightened patient safety [10, 12]. Additionally, intelligent automation enables laboratory staff to focus on complex interpretive and troubleshooting activities, thereby enhancing productivity and diagnostic accuracy. Essentially, ML enhances traditional QC methods by integrating decision-support intelligence into the analytical workflow [19]. Nonetheless, various factors need to be examined prior to the widespread adoption of ML-driven QC frameworks. Establishing uniformity in the processes of model development, validation, and reporting is crucial for achieving reproducibility and comparability of algorithms across different laboratories [10, 18]. The integrity of data is essential, as any inconsistencies in data entry, lack of complete metadata, or variations before analysis can undermine the learning process of models and their interpretability. Moreover, the potential dependence on algorithmic results without human supervision, along with ongoing concerns regarding regulatory compliance and data ethics, presents both practical and ethical challenges to clinical application [20, 21]. The primary advantage of this study is its ability to combine pooled sera with machine learning algorithms, yielding a cost-effective, matrix-relevant, and adaptive quality control system. The TriPPS framework effectively identifies random, systematic, and trend-related errors, showcasing robust analytical sensitivity and practical applicability.

However, the results stem from simulated bias and controlled error conditions that utilize only two electrolytes, potentially failing to encompass the full spectrum of real-world analytical variability. Comprehensive validation across a variety of analytes, instruments, and laboratory settings is necessary to

establish the general applicability of this method. It is important to emphasize that the present validation was conducted under controlled, simulated bias and noise conditions to enable systematic performance comparison across algorithms. While this approach allows precise quantification of detection lag and sensitivity, real-world analytical disturbances, such as reagent lot shifts, calibration instability, or instrument maintenance effects, may exhibit more complex patterns and require prospective validation. Moreover, the aspects of long-term stability, operator variability, and reagent-lot effects have not been thoroughly evaluated and require further exploration before routine application.

A logical next phase of investigation would include prospective validation across multi-analyte panels, multiple instrument platforms, and extended monitoring periods incorporating natural laboratory perturbations. Such expansion would determine robustness across different analytical principles and operational environments. Practical implementation would require integration within laboratory middleware or LIS environments capable of automated data streaming and algorithm execution in parallel with conventional QC modules. Transparent audit trails, version control of models, and predefined override mechanisms would be necessary to align with regulatory expectations for AI-assisted diagnostic systems. By focusing on these elements, we can ensure the secure and efficient integration of AI-powered quality control, allowing laboratories to transition from a reactive to a predictive operational framework.

Conclusion

The Triple-Point Pooled Sera (TriPPS) Quality Control system demonstrates how the integration of in-house pooled sera with machine learning algorithms can significantly enhance the detection of analytical errors in clinical laboratories. The integration of k-Nearest Neighbour, Isolation Forest, and Gaussian Process Regression allows the framework to effectively identify random, systematic, and trend-related deviations with minimal lag. This method provides a budget-friendly, relevant, and flexible solution that enhances conventional internal quality control practices, facilitating a shift from identifying errors after the fact to implementing proactive, data-informed laboratory monitoring. Extensive validation involving a variety of analytes and real-time clinical datasets will be crucial for positioning TriPPS as a fundamental element of smart quality assurance in laboratory medicine.

Ethical Approval

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. As only anonymized, discarded patient serum samples were used for pooled preparation, without any direct patient involvement or identifiable data, the Institutional Ethics Committee of the Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, waived the requirement for formal ethical approval.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this study. No financial, personal, or professional relationships influenced the conduct or reporting of this research.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. All data have been anonymized to protect patient confidentiality and comply with institutional data governance policies.

Author Contributions (CRediT Statement)

PD: Conceptualization, Writing – Review & Editing, Supervision. SN: Methodology, Software, Formal Analysis, Writing – Original Draft, Project Administration. SR: Investigation, Data Curation, Resources. BKK: Investigation, Data Curation, Resources. CRP: Investigation, Data Curation, Resources. DP: Investigation, Data Curation, Resources. TR: Investigation, Data Curation, Resources.

All authors reviewed and approved the final version of the manuscript.

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