Research Article

Analyzing the Utility of Pooled Sera as Internal Quality Control for Immunoassay Parameters by an EWMA-backed Statistical Mechanism (e-PSQC): A Comparison with Commercially Available Material and Westgard Rules

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Article Info

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Abstract

Background: Internal Quality Control (IQC) is a cornerstone of clinical laboratory operations, ensuring reliability in diagnostic testing. Commercial IQC materials, though effective, pose challenges of high cost, limited availability, and susceptibility to matrix effects. Pooled sera (PS), derived from discarded patient samples, offer a cost-effective alternative. However, the stability and performance of pooled sera as IQC material in immunoassays need robust evaluation, particularly when combined with advanced statistical tools like Exponentially Weighted Moving Average (EWMA).

Objective: To evaluate the utility of pooled sera as IQC material for immunoassay parameters using the EWMA statistical approach and compare its performance against commercially available IQC materials.

Method: A study was conducted in the clinical biochemistry laboratory at AIIMS Bhubaneswar. Pooled sera were prepared from discarded patient samples, aliquoted, and stored at -5°C. Five immunoassay parameters (Free T3, Free T4, TSH, Cortisol, and Ferritin) were monitored over 60 days using both pooled sera and commercial IQC materials. EWMA charts with a 7-day smoothing window were employed to assess error detection. Performance metrics, including Mean Squared Error (MSE), Root Mean Squared Error (RMSE), and Concordance Correlation Coefficient (CCC), were analyzed. Statistical significance was set at $p<0.05$.

Results: The EWMA analysis of pooled sera (ePSIQC) closely mirrored commercial IQC performance. Concordance was significant for all parameters except Free T4. The ePSIQC method demonstrated superior early error detection compared to traditional Westgard Multirules. Pooled sera remained stable throughout the study duration, with deviations observed only in a few instances.

Conclusion: Pooled sera, combined with EWMA, provides a cost-effective, stable, and reliable alternative to commercial IQC materials for immunoassays. The enhanced error detection capability of EWMA strengthens laboratory quality control, offering a viable solution for resource-limited settings.

Introduction

Clinical laboratories must have proper Quality Control (QC) since they supplement the highest laboratory performance standards, support accurate medical diagnosis and patient treatment, and strengthen the healthcare system. These materials comprise analytes of known concentrations that are measured by the laboratory, ideally in concentrations close to the decision limits where a medical decision is required [1,2]. They resemble human serum, plasma, blood, urine, and cerebrospinal fluid. The substance may be liquid (ready to use) or freeze-dried (lyophilised) [3]. On the day of the test, at least two levels of quality control should be done, regardless of the size of the lab. Two-level controls should be done during peak hours, and then one level should be done every eight hours if the lab is open throughout [4].

The need for internal QC (IQC) materials to monitor laboratory performance has grown as clinical laboratories have become more automated [5,6]. This is integral for monitoring and assessing the analytical processes that yield patient results. The primary objective is to identify and evaluate errors across the pre-analytical, analytical, and post-analytical phases before results are reported. Regular implementation of quality control materials is essential for ensuring the reliability of test outcomes. By routinely analysing and applying statistical process control, laboratories can uphold accuracy in patient testing. These results are deemed acceptable when they fall within the defined error limits and unacceptable when they exceed these boundaries, indicating significant errors [7–9]. Commercial control serums derived from animal or human serum are commonly used in clinical chemistry laboratories. QC materials must be homogeneous, stable, and non-infectious for extended storage periods. To mitigate matrix effects and facilitate efficient production, a pooled serum is typically prepared in bulk, aliquoted, and subsequently distributed to laboratories participating in an External Quality Assessment Scheme (EQAS) program [10]. However, limitations include bottle-to-bottle variations, reconstitution errors (e.g., temperature and solvent used), over-dilution or under-dilution, reconstitution duration, vigorous shaking, light exposure and compositional differences [11]. The routine use of commercial QC materials is often economically unfeasible for many developing countries due to their high cost and limited availability [5,12].

According to Good Laboratory Practice and Health Laboratory Practice Guidelines, laboratories may also use self-prepared control materials, such as serum pools, as alternatives [13]. Pooled serum (PS), a blend of human serum from clinical laboratory samples, closely resembles patient samples and reduces reconstitution errors typical of lyophilised commercial IQC [5,14,15]. While it's a cost-effective alternative, it's essential to evaluate the effectiveness and stability of pooled serum as a substitute for commercial quality control materials for routine biochemical parameters [14].

The procedure of quality control is statistical. The values of the control material within the specified upper and lower limits are shown on QC charts. Westgard rules can be used to discover errors during the analytical stage of sample processing [16]. Plotted on a Levy Jennings Chart, it is used to estimate the magnitude of various analytical errors, both systematic and random [17].

One common type of control rule in the workplace is the exponentially weighted moving average (EWMA) rule for internal quality control. The rule aims to accurately estimate systematic errors by combining control measures from earlier runs with control data from the present run. As a result, the EWMA rule is more sensitive than conventional QC methods that only assess the current run's control data, allowing it to detect small but consistent shifts or gradual trends much earlier. Because the influence of the earlier observations is exponentially decreasing, control measurements taken multiple runs before the current run only make a very small contribution, which makes sense intuitively [18,19].

This study aims to evaluate the effectiveness of using pooled serum samples in combination with the Exponentially Weighted Moving Average (EWMA) method for internal quality control (IQC). By integrating the inherent stability of pooled sera with the sensitivity of EWMA in detecting subtle analytical shifts or trends, we seek to determine whether this approach can enhance error detection in comparison to conventional methods. The performance of this strategy is assessed against that of commercially available quality control materials and standard Westgard rules. We hypothesize that the combined use of pooled sera and EWMA will provide earlier and more reliable identification of systematic errors, thereby improving the overall robustness of laboratory quality control practices.

Methodology

The Pooled Sera Quality Control (PSQC) analysis was conducted in the clinical biochemistry laboratory under the Department of Biochemistry at AIIMS Bhubaneswar. Ethical approval was obtained from the Institutional Ethical Committee.

Test parameters and instrument

A total of five routine parameters measured by an immunoassay, i.e., Free T3, Free T4, TSH, Cortisol and Ferritin, processed in Siemens Advia Centaur XP, were analysed by both commercial QC material as well as Pooled Sera Quality Control (PSQC). The sample load was around 500 tests/day. Therefore, the PSQC was processed around the midday mark for these parameters, approximating around 250 samples.

Commercial Quality Control Material

The quality control material used for day-to-day analysis was from BioRad. Two levels of Quality Control, Level 1 and Level 2 Lypocheck Immunoassay Plus Control.

Preparation of pooled sera and storage

The estimated requirement of sera for the entire parameter was around 500µL; for 60 days of processing, 30mL of pooled sera was required. The pooled sera utilized for this study were prepared on a single day (Day 0) from residual and discarded patient serum samples obtained in the clinical biochemistry laboratory. Based on the patient reports, only those samples that were in or close to the reference ranges were included in the process. Samples exhibiting apparent haemolysis, lipemia, icterus, or inadequate volume were eliminated to preserve analytical integrity. While individual testing of each sample for infectious pathogens, including HIV, Hepatitis B, and Hepatitis C, was not performed due to the retrospective and pooled methodology, rigorous compliance with universal precautions was maintained throughout the procedure. This encompassed the utilization of personal protective equipment (PPE) including gloves, lab coats, and face shields, in conjunction with appropriate hand hygiene and disinfection methods in accordance with institutional biosafety norms. After collecting a sufficient volume of pooled sera (30 mL), it was thoroughly mixed to guarantee uniformity. The pooled material was aseptically divided into sixty sterile 1 mL microcentrifuge tubes utilizing calibrated micropipettes to prevent contamination and volume inconsistencies. The aliquots were promptly stored at –5°C in a designated freezer container to maintain analyte stability throughout the 60-day quality control assessment period.

Processing of pooled sera

The PSQC was run as a patient sample, once every day for 60 days, using the hormone assay for the five parameters, and the values were noted. The values of Level 1 and Level 2 QC were also noted for this period.

Westgard Rules and Multirules

The Westgard Rules and Multirules were applied to the QC data. 1-2s was considered as a warning, and 1-3s was considered as run rejection. 2-2s, 3-1s, 4-1s, and NX rules were markers of systemic error and R-4s was taken as marker for random error.

EWMA

Exponentially Weighted Moving Average was applied to the measured value of PSQC (e-PSQC). A 7-day smoothing was used for EWMA with a λ of 0.25. The formula for the moving average estimation was:

EWMA(t) = 0.25 * X(t) + 0.25 * 0.75 * X(t-1) + 0.25 * 0.75^2 * X(t-2) + 0.25 * 0.75^3 * X(t-3) + 0.25 * 0.75^4 * X(t-4) + 0.25 * 0.75^5 * X(t-5) + 0.25 * 0.75^6 * X(t-6), where t =7 days.

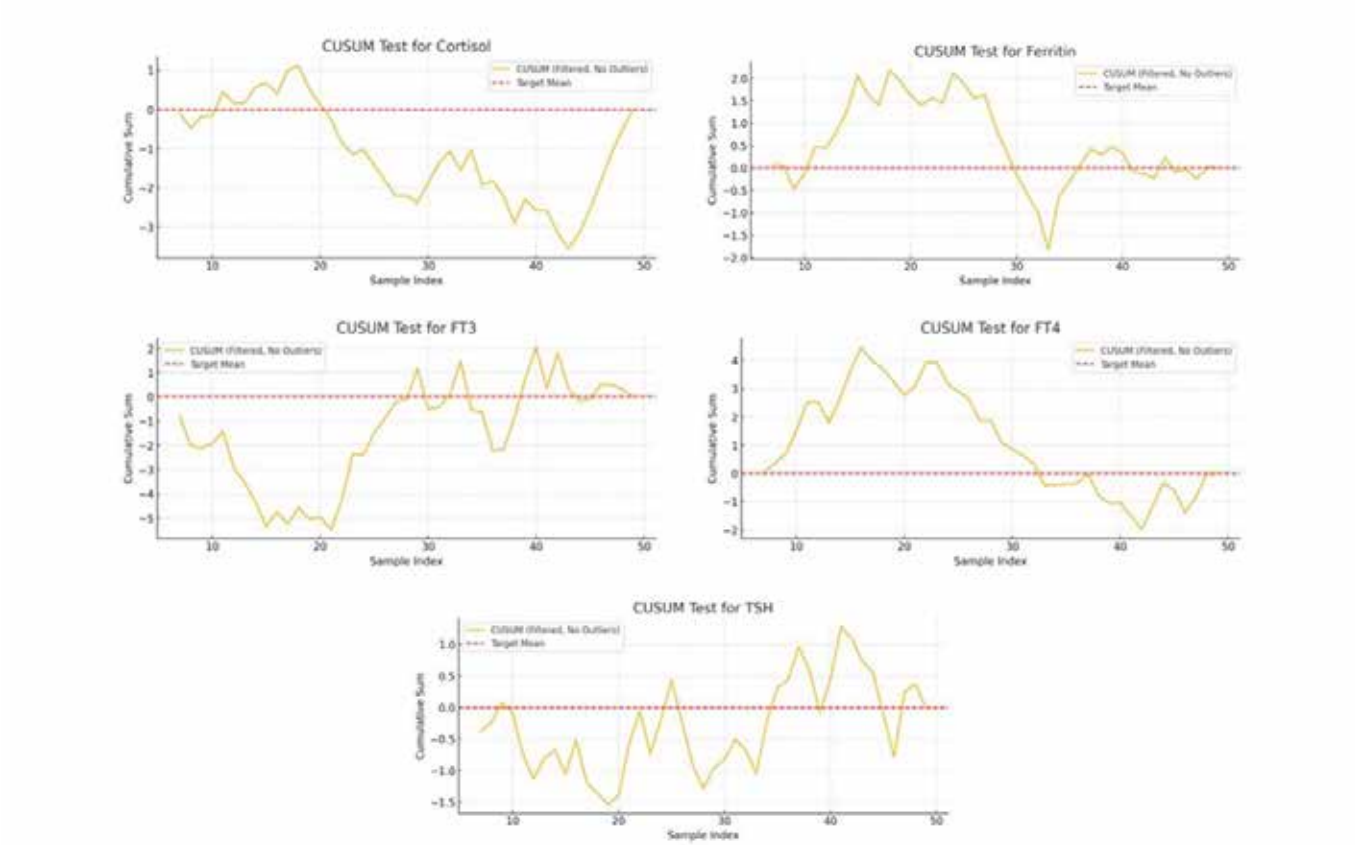
Statistical Analysis

The Westgard violations were determined using a custom-made MS Excel Worksheet based on the mean and SD of the analyte, as well as the QC run value for the given day. The stability of the Pooled Sera was determined by subtracting the days on which there were errors in the instrument and, for the remainder of the days, applying a CUSUM test. The agreement between the QC material and PSQC was determined based on evaluation metrics like Mean Squared Error (MSE), Root Mean Squared Error (RMSE), and Mean Absolute Deviation (MAD), as well as by Concordance Correlation Coefficient (CCC) analysis. A p-value of less than 0.05 was considered significant.

Results

The 7-day smoothed EWMA value from day 7 to day 60 was compared to that of the commercial internal QC run. The stability of the pooled sera was determined by subjecting it to the CUSUM test. For Cortisol and FT3, there was almost no deviation, whereas for other parameters, there were a few deviations around the mean, but those were not significant to disregard the sera. The CUSUM test result has been summarised in Figure 1.

Figure 1: CUSUM Chart for the Immunoassay parameter's PSIQC values on correct IQC days.



The deviation in the PSQC was well aligned with the deviations in the IQC material used. The concordance analysis showed a close agreement between L1 and L2 QC and the ePSIQC, except for FT4, where it was insignificant. The same has been tabulated in Table 1.

Table 1: Concordance Analysis between IQC and ePSIQC based on Z-Score.

Test	L1 CCC	L1 p-value	L2 CCC	L2 p-value
Cortisol	0.863	<0.001	0.726	<0.001
Ferritin	0.872	<0.001	0.657	<0.001
FT3	0.439	0.005	0.414	0.009
FT4	0.139	0.825	0.124	0.862
TSH	0.834	<0.001	0.825	<0.001

To evaluate the performance of ePSIQC compared to IQC, we calculated the evaluation metrics based on the corresponding measurements. This has been tabulated in Table 2. The EWMA calculated values and the L1 and L2 values of IQC were plotted on an LJ Chart, and the errors encountered were highlighted. The ePSIQC method accurately predicted the errors before Westgard Rules could make out the errors. This is shown in Figure 2.

Figure 2: EWMA-PSIQC and IQC L1 and L2 values on a standard LJ Chart.

Table 2: Performance Evaluation Metrics comparing ePSIQC to IQC by Z-Score deviation.

Test Analyte	IQC Level 1			IQC Level 2		
	MSE	RMSE	MAD	MSE	RMSE	MAD
Cortisol	0.02	0.05	0.04	0.02	0.05	0.04
Ferritin	0.03	0.05	0.04	0.04	0.07	0.06
FT3	0.20	0.14	0.11	0.13	0.11	0.09
FT4	0.24	0.15	0.14	0.31	0.18	0.16
TSH	0.04	0.06	0.05	0.04	0.07	0.05

Discussion

In our study, we tried to answer the research question in a three-fold pattern: If PS will be stable for immunoassay parameters over some time; If PS can be used as a supplement to Commercial QC material in the clinical laboratory; and whether combining PS with EWMA provides a robust pre-emptive mechanism to the Westgard Multirules.

The primary concern that remains in the usage of pooled sera is its stability once the material has been prepared for the parameters over the analysed days. In our case, for all parameters except FT4, the CUSUM measures a stable pooled sera parameter set. Though it is well set that Immunoassay parameters rarely deviate over some time, as reported by Westgard, they can be a practically viable supplemental material [20]. A similar study by Devi and Nagar illustrated the stability of PS over a period of 50 days when tested for routine biochemical parameters with no significant statistical deviation from the concentration mean as measured on Day 1 [14]. Kachhawa et al. also demonstrate the same where the PS stability is characteristically maintained throughout the study duration [21].

Once the stability was determined, the pooled sera measurement was coupled with EWMA to assess its effectiveness against IQC. It was shown that the EWMA

method closely follows both the levels of the IQC with respect to the Z-Score, thus proving its efficacy as an alternate QC material. This has been previously demonstrated in multiple previous studies, which returned with a similar conclusion [10]. Also, the more important finding of our study was that the deviation of EWMA was significantly earlier than that of Westgard Multirules and, therefore, could handle both systemic and random errors better than the conventional methods.
There were a few limitations to our study owing to the fact that multiple pooled sera, over a long period of time, could have a better result; also, rather than only assessing immunoassays, routine biochemical tests could have been included as they provide more complexity due to colorimetric test.
However, our study excelled at combining the statistically robust method of EWMA with a cost-conserving and robust alternative of pooled sera to formulate a 7-day smoothened ePSIQC that was significantly competent and, at times better, the commercially available and processed internal quality control in the laboratory.

Conclusion

Pooled serum Internal Quality Control (IQC) offers a practical, affordable, and flexible alternative to commercially available IQCs, especially for laboratories with financial constraints or limited access to commercial quality control materials. The implementation of Exponentially Weighted Moving Average (EWMA) as a predictive tool in conjunction with Westgard rules on a Levey-Jennings chart greatly improves the early detection of quality control errors, fostering a proactive approach to laboratory quality management. By identifying subtle shifts in control data that may indicate potential rule violations, EWMA enhances the quality control process, leading to better error detection and correction. This predictive capability helps laboratories uphold high analytical standards and reduces the risk of quality control breaches, ultimately reinforcing the reliability and accuracy of diagnostic testing. Combining both methods leads to a robust mechanism for early error detection, thus helping the laboratory, clinicians, and the patients.

CRediT Author Statement

PD: Conceptualization, Investigation, Writing-Reviewing & Editing, Supervision, Project Administration. SN: Conceptualization, Methodology, Software, Formal Analysis, Data Curation, Visualization. TR: Data Curation, Writing-Original Draft, Visualization.

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