Research Article

The efficacy of in-house prepared pooled sera compared to commercially acquired control serum for internal quality control at the Nkwen District Hospital laboratory.

Nfor Omarine Nlinwe[1] and Diom Loreen Ndum[1]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences, The University of Bamenda.

Abstract

The rise in automation across various laboratory processes has led to a substantially increased demand for quality control materials for performance monitoring. This study was designed to assess the efficacy of in-house prepared pooled sera with respect to the commercially acquired control serum, for internal quality control at the Nkwen district hospital laboratory.

Serum samples were pooled from ten voluntary blood donors who tested negative for HIV, HCV and HBsAg infections. Lyophilized commercial control sample was reconstituted and aliquots were prepared from both the pooled serum and the reconstituted commercially acquired control samples. The aliquots were stored at -20°C. Six biochemical parameters; creatinine, urea, aspartate aminotransferase, alanine aminotransferase, potassium ions and sodium ions were analyzed for a period of three months.

There were statistically significant differences in the mean values, standard deviation and coefficient of variation, between the pooled serum and the commercially acquired serum, all through the three months study period. Less variation was reported in the pooled serum measurements, all through the study period, suggesting higher precision.

In-house prepared pooled serum was found to be a superior alternative to the commercially acquired control sample for the measured parameters at the Nkwen District Hospital laboratory.

Key words: Pooled serum; Control samples; Biochemical parameters; Quality Control

Received: 23/02/2025

Accepted: 29/03/2025

DOI: https://dx.doi.org/10.4314/jcas.v21i3.2

Résumé

L'automatisation croissante des différents processus de laboratoire a entraîné une augmentation substantielle de la demande de matériel de contrôle qualité pour le suivi des performances. Cette étude visait à évaluer l'efficacité des sérums poolés préparés en interne par rapport au sérum témoin commercial, pour le contrôle qualité interne au laboratoire de l'hôpital du district de Nkwen.

Des échantillons de sérum ont été poolés auprès de dix donneurs de sang volontaires, négatifs aux tests VIH, VHC et HBsAg. Un échantillon témoin commercial lyophilisé a été reconstitué et des aliquotes ont été préparées à partir du sérum poolé et des échantillons témoins commerciaux reconstitués. Les aliquotes ont été conservées à -20 °C. Six paramètres biochimiques : créatinine, urée, aspartate aminotransférase, alanine aminotransférase, ions potassium et ions sodium ont été analysés pendant trois mois.

Des différences statistiquement significatives ont été observées dans les valeurs moyennes, l'écart type et le coefficient de variation entre le sérum groupé et le sérum commercial, tout au long des trois mois de l'étude. Une variation moindre a été signalée dans les mesures du sérum groupé, tout au long de la période d'étude, ce qui suggère une plus grande précision.

Le sérum groupé préparé en interne s'est avéré être une alternative supérieure à l'échantillon témoin acquis commercialement pour les paramètres mesurés au laboratoire de l'hôpital du district de Nkwen.
Mots clés : Sérum groupé ; Échantillons de contrôle ; Paramètres biochimiques ; Contrôle qualité
This text was translated from English to French using Google Translate and proofread by the Editor

Introduction

Evidence-based clinical guidelines indicate that at least 80% of guidelines focused on establishing a diagnosis or managing a disease necessitating laboratory testing (Badrick, 2021). As clinical laboratories automate more processes, the demand for quality control (QC) material has risen significantly for performance monitoring. QC is vital in all clinical laboratories, ensuring high standards, aiding accurate disease diagnosis and patient care, ultimately enhancing the healthcare system as a whole (Kanagasabapathy et al., 1996). Following NABL (National Accreditation Board for Testing and Calibration Laboratories) guidelines, QC should be performed at least twice daily (Dasgupta et al., 2013; Westgard et al., 2016).

A study determined the efficacy of pooled serum in comparison with commercially acquired control for internal quality control. They reported that using in-house quality control prepared from combined serum (for the following parameters: plasma glucose, blood urea, serum creatinine, total cholesterol, triglycerides (TGL), high-density lipoprotein, calcium, total protein, albumin, total bilirubin, ASAT, ALAT, ALP, amylase) proved superior to the commercial internal QC (Kulkarni et al, 2020). Results of some other findings equally reported that pooled serum demonstrates stability in biochemical parameters due to minimal matrix effects, resulting in less variation within the in-house IQC (Devi et al., 2023; Khatri et., 2013). Equal number of analytical errors were however observed for both commercially acquired and pooled sera for ALP, urea, sodium, potassium, and chloride but, there were significant differences between the mean values of bilirubin, ASAT and ALAT, on the first and last day of analysis **(**Devi et al., 2023**)**. Daily runs were done for a month and the mean levels of 12 biochemical analytes of the pooled sera were found to be within normal physiological range (Khatri et al., 2013).

The mean, coefficient of variation (CV %), and standard deviation for cholesterol, triglycerides, glucose, ASAT, ALAT, calcium, alkaline phosphatase, and amylase were found to be significantly different between the two groups (Jiskani et al., 2021). Less matrix effect was found in pooled serum making it a better internal quality control material than commercially acquired serum (Jiskani et al., 2021). With respect to duration of storage of the control materials, lyophilized serum was found to be stable for glucose, BUN, ALAT, total bilirubin and protein after storage at 2–8°C for seven months and at -20°C for nine months (Jamtsho, 2013). Likewise, when pooled sera was utilized for up to 6 months at -20°C storage, the concentration of most parameters did not significantly change, except for ALAT and bilirubin (Prasad et al., 2019).
To effectively monitor inaccuracy and imprecision, stable commercially acquired sera from the same lot is required through the period of envisioned use. However commercial materials could have inconsistent concentrations between vials and errors may arise during reconstitution (Kanagasabapathy et al., 1996). The method selected for pooling in this study was informed by the preparation technique for unmodified or minimally processed fresh frozen human sera. Results from studies using this method showed that the pooled sera exhibited significant stability

for up to 18 routine biochemical parameters, indicating that it acts as an effective and stable alternative for internal quality control material (Devi et al., 2023; Henriksen et al., 2004). Thus, creating and utilizing in-house quality control serum would significantly reduce costs related to laboratory reagents. Moreover many countries find it economically unfeasible to rely solely on commercial control material due to either unavailability or high costs (Kulkarni et al., 2020). Therefore this study was designed to assess the efficacy of in-house prepared pooled sera with respect to the commercially acquired control serum, for internal quality control at the Nkwen district hospital laboratory.

Materials and Methods

The study lasted for three months, from June to August 2022, and was conducted at the laboratory of Nkwen District Hospital. 3 mL of venous blood was obtained from ten apparently healthy adult voluntary blood donors who tested negative for HIV, HCV, and HBsAg infections. "Apparently healthy" because they exhibited no visible signs of illness or disease during the blood collection. The plain red-top test tubes without any additives were used for blood collection. Blood was left to clot at room temperature for 30 to 60 minutes and centrifuged. The clotted blood samples were visually examined and confirmed to be free from hemolysis, lipemia, or icterus, as indicated by the absence of red, pink, yellow, or amber colors, as well as the lack of a milky or cloudy appearance in the serum. 1000µL of serum was collected from each donor, pooled and thoroughly mixed in a sterile glass jar at low temperatures using an ice bath. That is, the serum from donors were added gently and gradually to the sterile container to facilitate even mixing. A sterile spatula was then employed to carefully mix the pooled sera using a folding motion to avoid the formation of bubbles and minimize shear stress on the proteins. Sixty five aliquots of 150µL each of the thoroughly mixed pooled sera were dispensed into polypropylene vials, and frozen at -20°C without any additional processing. The lyophilized commercial control samples were reconstituted and sixty five aliquots of 150µL were also prepared and frozen at -20°C. Alongside the commercial control sample, an aliquot of pooled serum was analyzed each day, for a 20 day period in a month (June to August 2022).

The following parameters were measured: blood urea, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALAT), potassium and sodium ions. The first 20 values for each parameter were used to calculate the mean, standard deviation and coefficient of variation for the pooled serum while those values were provided for the commercially acquired control sample. These values were used to establish the Levey-Jennings (L-J) charts. The Levey–Jennings chart was used to plot both QC values and the Westgard multirules were applied. Protective and remedial measures were taken for any outliers in the values. Data were entered in Microsoft Excel sheet and analyzed using Graphpad prism version 8.4.2. Results were presented as mean ± SD and CV%. The unpaired t test with Welch's correction was used to determine the difference between commercial IQC and pooled serum IQC. Statistical significance was considered at a 5% level of significance.

Results

The mean, standard deviation (SD) and percentage coefficient of variation (CV %) were calculated for each of the three months. When compared with the commercially acquired control serum, there were statistically significant differences with the in-house prepared control serum for all the biochemical parameters under study (Creatinine, urea, ASAT, ALAT, K^+ and Na^+), throughout the three months study period.

For creatinine, the standard deviation (SD) values for the in-house quality control (QC) samples were consistently significantly lower than those of the commercial QC samples during the first and second months, while they were equal in the third month. The mean values of creatinine for both control samples fell within the normal ranges. However, the coefficient of variation (CV) values for the in-house QC samples were higher than those of the commercial QC samples across the entire evaluation period.

In the case of urea, the SD values for the in-house QC samples were significantly lower than those of the commercial QC samples throughout the three-month period. The mean values of urea for both control samples were outside the normal ranges. Similarly, the CV values for the in-house QC samples were lower than those of the commercial QC samples during the entire period. (See table 1).

Table 1: Comparison of Commercial and In-house internal quality controls for Creatinine and Urea.

Months	Parameters	Normal ranges	Mean ± SD		CV%		*p* -Value
Month 1			Commercial QC	In-house QC	Commercial QC	In-house QC	
	Creatinine (mg/dL)	0.6 to 1.3	1.2 ± 0.14	0.77 ± 0.13	12	16	<0.0001*
	Urea (mg/dL)	7 to 20	44 ± 4.8	22 ± 1.8	11	8.5	<0.0001*
Month 2	Creatinine (mg/dL)	0.6 to 1.3	1.2 ± 0.13	0.83 ± 0.12	11	14	<0.0001*
	Urea (mg/dL)	7 to 20	43 ± 4.3	22 ± 1.5	10	7	<0.0001*
Month 3	Creatinine (mg/dL)	0.6 to 1.3	1.2 ± 0.13	0.77 ± 0.13	10	16	<0.0001*
	Urea (mg/dL)	7 to 20	43 ± 4	22 ± 1.8	9.2	8.5	<0.0001*

* $P < 0.05$.

For ASAT, the standard deviation (SD) values for the in-house quality control (QC) samples were lower than those for the commercial QC samples throughout the entire three-month study period. Additionally, the mean values for both sample types remained within the normal range. In terms of the coefficient of variation (CV), the in-house QC samples consistently exhibited lower CV values compared to the commercial QC samples over the three-month period.

Regarding ALAT, the SD values for the in-house QC samples were also lower than those for the commercial QC samples for the entire study period. While the mean values for the in-house QC samples consistently fell within the normal range, the mean values for the commercial QC samples remained outside of the normal range. However, the CV values for the in-house QC samples were consistently higher than those of the commercial QC samples (see Table 2).

For K+, the standard deviation (SD) values for the in-house quality control (QC) samples were lower than those of the commercially acquired samples during the first and second months, but were higher in the third month. While the mean values for the commercial QC samples remained within the normal range, the mean value for the in-house control samples fell within the normal

Table 2: Comparison of Commercial and In-house internal quality controls for ASAT and ALAT.

Months	Parameters	Normal ranges	Mean ± SD		CV%		*p* -Value
Month 1			Commercial QC	In-house QC	Commercial QC	In-house QC	
	ASAT (U/L)	15 to 40	31 ± 6	22 ± 2.8	19	13	<0.0001*
	ALAT (U/L)	10 to 40	51 ± 5	26 ± 4.6	9.9	18	<0.0001*
Month 2	ASAT (U/L)	15 to 40	30 ± **5.7**	22 ± **1.7**	19	7.9	<0.0001*
	ALAT (U/L)	10 to 40	49 ± **5**	26 ± **3.5**	10	13	<0.0001*
Month 3	ASAT (U/L)	15 to 40	30 ± **4.3**	22 ± **2.8**	14	13	<0.0001*
	ALAT (U/L)	10 to 40	49 ± **5.7**	26 ± **4.6**	12	18	<0.0001*

* P < 0.05. **Abbreviations:** ASAT, Aspartate aminotransferase; ALAT, alanine aminotransferase.

range only during the second month. The coefficient of variation (CV) values for the in-house QC samples were lower than those of the commercial QC samples only during the first and second months.

For Na+, the SD values for the in-house QC samples were consistently lower than those of the commercial QC samples throughout the three-month period. Meanwhile, the mean values for the in-house QC samples were consistently outside the normal range, while those for the commercial QC samples remained within the normal range. The CV values for the in-house QC samples were consistently lower than those of the commercial QC samples (see Table 3).

Table 3: Comparison of Commercial and In-house internal quality controls for K^+ and Na^+.

Months	Parameters	Normal ranges	Mean ± SD		CV%		*p* -Value
Month 1			Commercial QC	In-house QC	Commercia l QC	In-house QC	
	K^+ (mmol/L)	3.5 to 5.0	3.7 ± 0.37	3.4 ± 0.32	10	9.3	0.0010*
	Na^+ (mmol/L)	135 to 145	141 ± 10	122 ± 4.9	7.2	4	<0.0001*
Month 2	K^+ (mmol/L)	3.5 to 5.0	3.8 ± 0.34	3.5 ± 0.28	9.1	8.2	0.0011*
	Na^+ (mmol/L)	135 to 145	142 ± **9.1**	120 ± **3.2**	6.4	2.6	<0.0001*
Month 3	K^+ (mmol/L)	3.5 to 5.0	3.8 ± 0.30	3.4 ± 0.32	8	9.3	<0.0001*
	Na^+ (mmol/L)	135 to 145	139 ± **7.7**	122 ± **4.9**	5.5	4	<0.0001*

* P < 0.05. K^+, Potassium ions; Na^+, Sodium ions.

Discussion

This study was out to determine the efficacy of pooled sera as an in house quality control sample with respect to the commercially acquired control sample. Both aliquot control samples were stored at -20°C and analyzed for 20 days each month over a period of 3 months. The results showed significant differences in the mean, SD and CV % values between the pooled sample and the commercially acquired sample, throughout the 3 month period. The mean values for the in-house prepared control sample were significantly lower than the mean values for the commercially control sample, throughout the three month period. For the first and the third months mean values for the in-house control sample were within the normal ranges for three (Creatinine, ASAT and ALAT), out of the six biochemical parameters measured. For the second month the mean values for the in-house control sample were within range for four (Creatinine, ASAT, ALAT and K^+), out of the six parameters measured. Despite being out of the normal ranges, mean values for K^+ and urea were close to the lower and the upper limits respectively, all through the study period. For the commercially acquired sample the mean values of four (Creatinine, ASAT, K+, and Na+) out of the six measured parameters remained within the normal range all through the study period. In contrast to the in-house control sample, the out-of-normal-range mean values for urea and ALAT were not near the upper limit of the normal range. This indicates that the mean values for the in-house control samples were generally closer to the normal ranges compared to the commercially acquired control sample. In line with findings from other studies pooled serum demonstrates more stability in these biochemical parameters probably due to minimal matrix effects, resulting in less variation within the mean values (DEVI & NEGI, 2023; Jiskani et al., 2021; Khatri et al., 2013; Prasad et al., 2019).

The SD values for the in-house prepared control samples were all less than that of the commercially acquired serum all through the three months period of analysis. Meanwhile the CV% values for the in-house QC samples were less than the values for commercially acquired control samples for urea, ASAT, K^+ and Na^+ for the first and second months. But for the third month CV% control values for the in-house prepared control sample were less for, urea, ASAT, and Na^+. CV% and SD control values for biochemical parameters were also found to be significantly different between pooled and commercially acquired control samples (Anggra WN, 2021 ; Jiskani et al., 2021) Although both metrics assess data dispersion, the main distinction is that coefficient of variation (CV) presents the standard deviation as a percentage of the mean, allowing for comparisons of datasets with varying scales. In contrast, standard deviation (SD) simply shows how much the data varies from the mean in its original units (Shechtman, 2013). Because the current data sets are measured on the same scale, the Standard Deviation (SD) values appear to be a more reliable choice for analyzing absolute variability, in contrast to the Coefficient of Variation (CV). Therefore the consistently lower standard deviation (SD) values for the in-house prepared control sample indicates reduced variability in its repeated measurements over the three-month study period compared to, the commercially acquired sample. Less variability in repeated measurements of the in-house control sample further indicates higher precision, signifying effective quality control processes.

Conclusion:

Internal quality control was done using six biochemical parameters; creatinine, urea, ASAT, ALAT, K^+ and Na^+. Pooled serum was used as the in-house prepared control sample in comparison with the commercially acquired control sample. Mean values for the pooled control sample were significantly lower than mean

values for the commercially acquired control sample. Less variation was mostly reported in the pooled serum measurements all through the study period, suggesting higher precision. Thus in-house prepared pooled serum appeared to be a better substitute for the commercially acquired control sample for creatinine, urea, ASAT, ALAT, K^+ and Na^+ at the Nkwen district hospital laboratory. Further study is recommended for other biochemical parameters in the same study area.

References

Anggra WN. (2021). Comparison between Pooled Sera Material and Commercial Serum on the Accuracy of Triglyceride *Atlantis Press., Check. In2nd Syedza Saintika International Conference on Nursing, Midwifery, Medical Laboratory Technology, Public Health, and Health Information Management (SeSICNiMPH 2021)*. 330-334. .

Badrick T. (2021). Integrating quality control and external quality assurance. *Clinical biochemistry. 95*: 15-27.

Dasgupta A, Wahed A. (2013). Clinical chemistry, immunology and laboratory quality control: a comprehensive review for board preparation, certification and clinical practice.

DEVI A, NEGI A. (2023). Pooled Sera as an Alternative to Commercial Internal Quality Control in Clinical Laboratories. *Journal of Clinical & Diagnostic Research. 17*(10).

Henriksen G, Pedersen M, Nørgaard I, Blom M, Blou L, Blaabjerg O, Uldall A. (2004). Minimally processed fresh frozen human reference sera: preparation, testing, and application to international external quality assurance. *Scandinavian journal of clinical and laboratory investigation. 64*(4): 293-308.

Jamtsho R. (2013). Stability of lyophilized human serum for use as quality control material in Bhutan. *Indian Journal of Clinical Biochemistry. 28*(4): 418-421.

Jiskani SA, Pirzado AA, Saleem MW, Naz L, Shaikh A, Atzaz N. (2021). Determination of Pooled Serum as a Better and Cheap Method for Internal Quality Control in Clinical Laboratory. *Linguistica Antverpiensia*, 2564–2571-2564–2571.

Kanagasabapathy A, Swaminathan S, Selvakumar R. (1996). Quality control in clinical biochemistry. *Indian Journal of Clinical Biochemistry. 11*: 17-25.

Khatri R, Shrestha P, Sinha J. (2013). Implementing self-sustained quality control procedures in a clinical laboratory. *Journal of Nepal Medical Association. 52*(189): 233-237.

Kulkarni S, Pierre SA, Kaliaperumal R. (2020). Efficacy of pooled serum internal quality control in comparison with commercial internal quality control in clinical biochemistry laboratory. *Journal of laboratory physicians, 12*(03): 191-195.

Shechtman O. (2013). The coefficient of variation as an index of measurement reliability. In Methods of clinical epidemiology *Springer Berlin Heidelberg., Berlin, Heidelberg:* 39-49.

Prasad P, Kumari R, Kumar R, Kumar S, Kumar U, Shekhar R. (2019). Stability of Frozen Pooled Serum for Use as Quality Control Material. *International Journal of Contemporary Pathology: 5*(1).

Westgard JO, Westgard SA. (2016). Quality control review: implementing a scientifically based quality control system. *Annals of clinical biochemistry: 53*(1), 32-50.