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Original Article

Biochemistry Section

Pooled Sera as an Alternative to Commercial Internal Quality Control in Clinical Laboratories

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ABSTRACT

Introduction: Internal Quality Control (IQC) is run to assess the day-to-day performance of an analytical process, ensuring the reliability of patient results. However, commercial internal quality materials can be expensive and inaccessible for many laboratories. Therefore, there is a need for an effective and economical alternative to commercial IQC.

Aim: To evaluate the effectiveness and stability of pooled sera as an IQC material.

Materials and Methods: A laboratory study was conducted over a period of 50 days, from January 10 to February 28, 2023, in the Department of Biochemistry at Dr. Rajendra Prasad Government Medical College (DRPGMC), Kangra, Himachal Pradesh, India. Serum samples (150 µL) were collected from 100 leftover samples over a span of 10 days. The serum was thoroughly mixed and distributed into 75 aliquots. Commercial IQC was also reconstituted and distributed into 75 aliquots. Both the commercial and pooled sera IQC were stored at -20°C. One aliquot of pooled sera was analysed daily for 16 routine biochemistry parameters, along with the commercial IQC, for the entire 50-day period. The results from both materials were interpreted using L-J chart and Westgard multirule. The stability of pooled sera for these routine parameters was assessed by comparing the mean values of the biochemical parameters on day 1 and day 50. Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0 software, and a paired t-test with a p-value <0.05 was considered statistically significant. The bias percentage for each pooled sera parameter was calculated and compared with the desirable bias for clinical significance.

Results: Analytical errors were observed during the study period for Alkaline Phosphatase (ALP), urea, sodium, potassium, and chloride. The nature and number of analytical errors observed with both materials were the same, with Westgard rule 1_{3s} detecting random errors for ALP and rule 2_{2s} detecting systematic errors for urea and serum electrolytes (sodium, potassium, and chloride). Difference in the mean value of alanine transaminase, aspartate transaminase, and bilirubin, on day 1 and day 50 was statistically significant with p-values 0.035, 0.04, and 0.024, respectively. When these parameters were assessed for clinical bias, they were found to be clinically insignificant.

Conclusion: Pooled serum is an effective and stable alternative IQC material for daily performance assessment of analytical processes. In-house IQC or pooled sera not only reduces the cost of quality control but also helps maintain continuity in the absence of commercial IQC, preventing any disruptions in the control system over an extended period.

Keywords: Alanine transaminase, Aspartate transaminase, Bilirubin, L-J charts, Pooled serum, Westgard rules

INTRODUCTION

The quality of a clinical laboratory is assessed using different quality indicators at various phases of the total testing process, including the preanalytical, analytical, and postanalytical levels [1,2]. Quality control material is utilised at the analytical level to reflect the variation occurring at that stage. In the laboratory, quality controls involve IQC and external quality control. IQC assesses the day-to-day intralaboratory variation [3], whereas external quality control evaluates interlaboratory variation [4] and is performed on a monthly basis. IQC plays a crucial role in monitoring laboratory variation and determining whether the observed variations are acceptable or not [5,6]. This information is vital in deciding whether to proceed with sample analysis and release the patient's report, thereby avoiding incorrect reporting. IQC is performed by running quality control material that should be homogeneous, stable, non infectious, and affordable. Quality control materials available in the market can be either lyophilized or in liquid form, and they can be assayed or non-assayed. Commercial quality control materials can be derived from human or animal sources. However, the use of commercial quality control materials has certain drawbacks, such as lot-to-lot variation in concentration, reconstitution-related errors (e.g., temperature and solvent used), over dilution or under dilution, reconstitution duration, vigorous shaking, and light exposure [5,6]. While these issues associated with commercial IQC use are not unavoidable, some can be mitigated. Matrix-related variation can be minimised by using human-origin quality control material, and reconstitution-related errors can be minimised by using liquid quality control material. Additionally, the problem of lot variation can be mitigated by purchasing quality control material from the same lot for an extended period of time. Despite being effective, commercial IQC materials are expensive. In resource-limited developing countries, many laboratories find commercial IQC economically unviable due to its cost. In such cases, pooled sera can serve as an alternative to commercial IQC. Pooled serum is a homogenised mixture of human serum that can be easily prepared using leftover samples in any clinical laboratory. Pooled serum has a matrix closer to that of the patient and avoids reconstitution-related errors observed with lyophilised commercial IQC. While pooled serum is a cost-effective and economical alternative to commercial IQC, this should not come at the expense of compromising overall laboratory quality. Therefore, it is necessary to assess the effectiveness and stability of pooled sera as an alternative to commercial quality control material for various routine biochemical parameters. This study aimed to evaluate the effectiveness and stability of pooled sera as an IQC material.

MATERIALS AND METHODS

A laboratory study as a quality control initiative was conducted in the Department of Biochemistry, Dr. Rajendra Prasad Medical College Kangra at Tanda, Himachal Pradesh, India, after obtaining approval from the Institutional Ethical Committee (IEC) (no. IEC/036/2022). The study was conducted over a period of 50 days, from January

10 to February 28, 2023. Informed consent was obtained from the patients, and 100 leftover serum samples after routine biochemistry analysis were collected.

Inclusion criteria: Leftover serum samples negative for Human Immunodeficiency Virus (HIV), Hepatitis B surface Antigen (HbsAg), and Hepatitis C Virus (HCV) were included in the study.

Exclusion criteria: Samples with significant haemolysis, lipemia, or icterus were excluded from the study.

Procedure

A 15 mL pooled serum was prepared by collecting 150 µL of leftover serum sample from 10 samples daily for 10 days and stored in a glass flask. After collecting the pooled sera, it was mixed and processed for homogenisation. Then, it was distributed into 75 aliquots, each containing a volume of 200 µL. No preservative was used. Commercial lyophilised IQC of ERBA NORM from ERBA Mannheim was reconstituted following the manufacturer's recommended precautions and distributed into 75 aliquots, each containing 200 µL. Both the pooled sera and commercial IQC were stored at -20°C until further processing. One aliquot of pooled sera was processed daily for 50 days along with commercial IQC for 16 routine biochemical parameters (Glucose, urea, creatinine, bilirubin, ALT, AST, ALP, protein, albumin, cholesterol, triglyceride, calcium, phosphorus, sodium, potassium, and chloride) on a tranasia XL-640 analyser using standard reagents. The initial 20 days' data were used to plot the L-J chart [7]. The values of pooled sera and commercial IQC were entered in this chart on a daily basis for 30 days and interpreted using the Westgard multirule [Table/Fig-1] [8,9]. To assess the stability of pooled sera, 16 parameters were run in repeats of 10 on day 1 and day 50.

Rule	Description
1_{2S}	One control observation exceeding the mean±2s; used as warning rule.
1_{3S}	One control observation exceeding the mean±3s; is a rejection rule that is sensitive to random error.
2_{2S}	Two consecutive control observations exceed the same 2s or the same mean minus 2s control limit. It is a rejection rule that is sensitive to systematic error. This rule can apply to a single level during two consecutive run or both the control levels in the same run.
R_{4S}	The range between the two consecutive values of same control level is greater than or equal to 4s where one value is plus 2s and other is minus 2s. It is rejection rule sensitive to random error.
4_{1S}	Four consecutive values exceeding the mean plus 1SD or mean minus 1SD. This can involve one or both the controls. It is a rejection rule that is sensitive to systematic error.
10_{x}	Ten consecutive observations falling on one side of the mean. This can apply to within run or across the run. It is a rejection rule that is sensitive to systematic error.

[Table/Fig-1]: Westgard Multirule [8,9].

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 20.0 software. The mean, standard deviation, and coefficient of variation were calculated for both materials using the data from the initial 20 days of the study. These values were then used to plot an L-J chart, where the control value (mean±3SD) was plotted on the y-axis and the date/time was plotted on the x-axis. The results of each parameter on day one and day 50 were presented as mean±SD. The mean values of the 16 parameters on day one and day 50 were compared using a paired t-test, with a p-value of <0.05 considered statistically significant. The bias percentage for each parameter was calculated using the formula (Bias %=$D_{50\ Mean}$-D1 mean/$D_{1\ mean}$×100) and compared with the desirable bias for each parameter to assess its clinical significance [10].

RESULTS

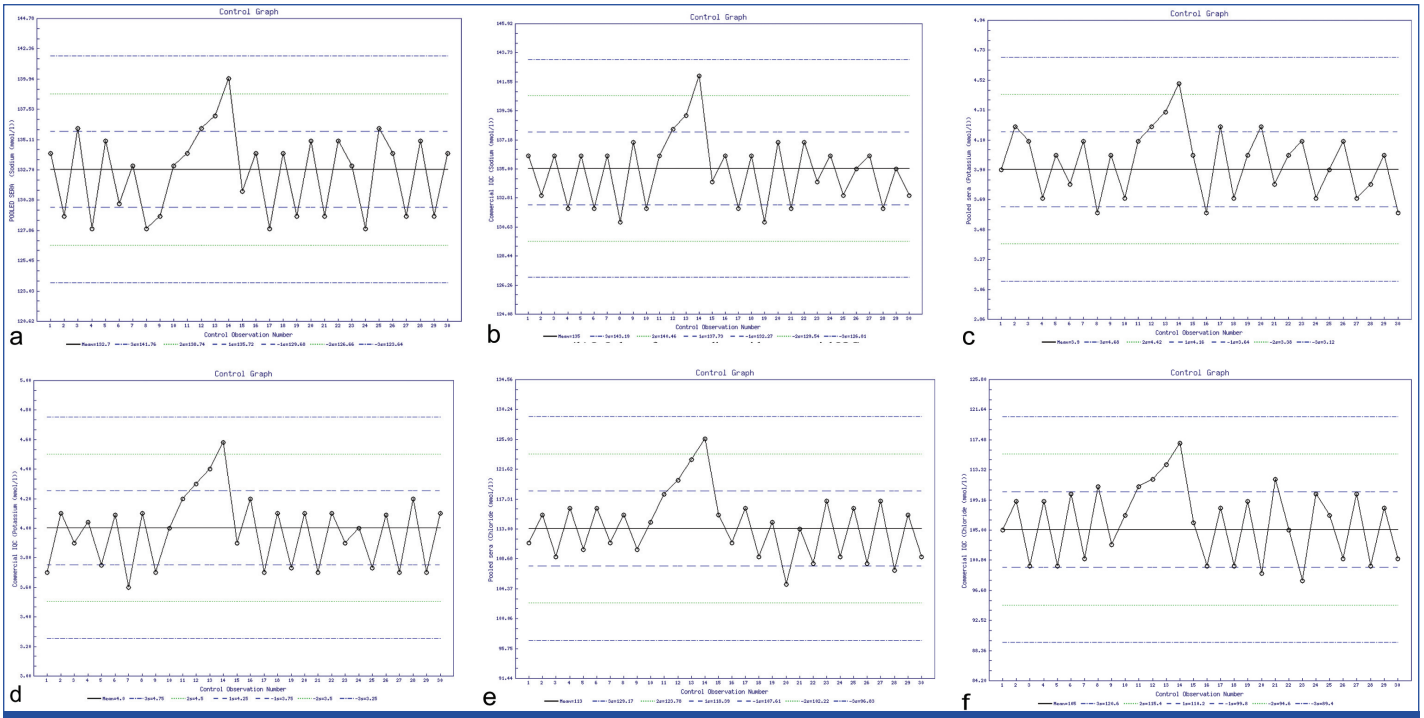
The mean and Standard Deviation (SD) of the initial 20 days' data were used to plot the L-J chart for pooled sera and commercial IQC [Table/Fig-2]. The mean±SD of the pooled sera data for routine parameters were as follows: glucose (109.4±5.79 mg/dL), urea (30.1±1.44 mg/dL), creatinine (1.04±0.10 mg/dL), total bilirubin (1.46±0.07 mg/dL), AST (30.4±3.42 U/L), ALT (27.6±2.18 U/L), Albumin (4.0±0.25 g/dL), cholesterol (164±6.52 mg/dL), triglyceride (140±5.39 mg/dL), calcium (7.9±0.45 mg/dL), phosphorus (3.8±0.31 mg/dL), sodium (132.7±3.02 mmol/L), potassium (3.9±0.26 mmol/L), and chloride (113±5.39 mmol/L). The commercial IQC data for routine parameters were as follows: glucose (89.7±5.48 mg/dL), urea (39.7±1.37 mg/dL), creatinine (1.23±0.12 mg/dL), total bilirubin (1.05±0.07 mg/dL), AST (35.2±3.91 U/L), ALT (38.5±2.11 U/L), ALP (128.4±9.64 U/L), total protein (5.56±0.34 g/dL), albumin (3.5±0.26 g/dL), cholesterol (141.7±6.59 mg/dL), triglyceride (132±5.32 mg/dL), calcium (7.6±0.45 mg/dL), phosphorus (4.0±0.32 mg/dL), sodium (135.0±2.73 mmol/L), potassium (4.0±0.25 mmol/L), and chloride (105±5.20 mmol/L). The pooled sera data for ALP, Na, K, Cl, and urea violated the rules, with one value of ALP being +3SD on day 9, values for each serum electrolyte (Na, K, and Cl) being +2SD on day 14, and one value for urea being +2SD on day 18. These analytical errors were consistent with the observations from the commercial IQC. The Westgard within-run rule was used for the final data interpretation. Root cause analysis was performed for each analytical error, and appropriate corrective actions were taken [Table/Fig-3]. The L-J chart for pooled serum and commercial IQC for serum electrolytes is provided in [Table/Fig-4]. There was no significant difference (p>0.05) in the mean value of most of the

Parameters	N	Pooled sera		Commercial IQC	
		Mean±SD	CV%	Mean±SD	CV%
Glucose (mg/dL)	20	109.4±5.79	5.34	89.7±5.48	6.1
Urea (mg/dL)	20	30.1±1.44	4.68	39.7±1.37	3.45
Creatinine (mg/dL)	20	1.04±0.10	9.61	1.23±0.12	9.75
T Bil (mg/dL)	20	1.46±0.07	4.8	1.05±0.07	6.7
AST (U/L)	20	30.4±3.42	11.2	35.2±3.91	11.1
ALT (U/L)	20	27.6±2.18	7.8	38.5±2.11	5.5
ALP (U/L)	20	99.2±8.30	8.4	128.4±9.64	7.6
Total protein (g/dL)	20	6.0±0.32	5.3	5.56±0.34	6.1
Albumin (g/dL)	20	4.0±0.25	6.2	3.5±0.26	7.4
Cholesterol (mg/dL)	20	164±6.52	3.9	141.7±6.59	4.6
TG (mg/dL)	20	140±5.39	3.8	132±5.32	4.0
Calcium (mg/dL)	20	7.9±0.45	5.6	7.6±0.45	5.9
Phosphorus (mg/dL)	20	3.8±0.31	8.1	4.0±0.32	8.0
Sodium (mmol/L))	20	132.7±3.02	2.27	135±2.73	2.0
Potassium (mmol/L)	20	3.9±0.26	6.7	4.0±0.25	6.2
Chloride (mmol/L)	20	113±5.39	4.7	105±5.20	4.9

[Table/Fig-2]: Pooled sera and commercial Internal Quality Control (IQC) parameter data used for L-J chart.

Parameter	QC	Run no.	Westgard rule	Within run rule	Cause	Corrective action
ALP	Pooled	9	1_{3S}	1_{3S}	Calibration factor out of limit	Fresh calibration run
	Commercial	9	1_{3S}			
Na	Pooled	14	1_{2S}	2_{2S}	Reagent pack deterioration	Reagent pack changed
	Commercial	14	1_{2S}			
K	Pooled	14	1_{2S}	2_{2S}		
	Commercial	14	1_{2S}			
Cl	Pooled	14	1_{2S}	2_{2S}		
	Commercial	14	1_{2S}			
Urea	Pooled	18	1_{2S}	2_{2S}	Reagent contamination	Reagent pack changed
	Commercial	18	1_{2S}			

[Table/Fig-3]: Rule violation observed and corrective action taken with pooled sera and commercial IQC.



[Table/Fig-4]: L-J chart where y-axis represent the control value and x-axis represent the day/number of observation. [Table/Fig-4a-f] are showing the upward treand with pooled sera and commercial IQC for serum sodium, potassium and chloride, respectively. a) L-J chart of serum sodium with pooled sera; b) L-J chart of serum sodium with commercial IQC; c) L-J chart of serum potassium with pooled sera; d) L-J chart of serum potassium with commercial IQC; e) L-J chart of serum chloride with pooled sera; f) L-J chart of serum chloride with commercial IQC.

pooled sera parameters between day one and day 50, except for AST (p-value 0.04), ALT (p-value 0.035), and bilirubin (p-value 0.024), where a significant decrease was observed on day 50. However, when the difference in these three parameters was assessed for clinical bias, it was found to be insignificant [Table/Fig-5].

Parameters	D_1 (Mean±SD)	D_{50} (Mean±SD)	Bias %	p-value <0.05	Desirable bias %
Glucose (mg/dL)	108.4±5.79	107.0±5.34	-0.64	0.52	2.30
Urea (mg/dL)	30.5±2.01	29.6±2.39	-2.95	0.25	5.56
Creatinine (mg/dL)	1.08±0.10	1.09±0.11	0.92	0.67	4.00
T Bil (mg/dL)	1.45±0.10	1.40±0.11	-3.44	0.024*	9.00
AST (U/L)	30.7±3.38	30.0±3.39	-2.28	0.040*	6.54
ALT (U/L)	26.6±2.72	25.2±2.38	-5.26	0.035*	11.48
ALP (U/L)	99.4±8.12	98.1±8.06	-1.30	0.10	6.72
Total protein (g/dL)	6.10±0.30	6.08± 0.30	-0.33	0.71	1.40
Albumin (g/dL)	4.04±0.25	4.0±0.28	-0.99	0.09	1.43
Cholesterol (mg/dL)	164.4±6.52	163.2±6.62	-0.72	0.06	4.10
TG (mg/dL)	140.1±5.39	139.5±5.50	-0.42	0.110	9.60
Calcium (mg/dL)	7.96±0.45	7.97±0.47	0.12	0.62	0.80
Phosphorus (mg/dL)	3.84±0.31	3.89±0.33	1.30	0.135	3.38
Sodium (mmol/L)	132.7±3.02	132.5±3.00	-0.15	0.349	0.20
Potassium (mmol/L)	3.93±0.26	3.95±0.28	0.50	0.056	1.80
Chloride (mmol/L)	113.5±5.39	113.2±5.61	-0.26	0.49	0.50

[Table/Fig-5]: Stability of pooled sera for biochemistry parameters.

DISCUSSION

The present study assessed the effectiveness and stability of pooled sera as an IQC material. The errors observed for pooled sera and commercial IQC were similar in number and nature, indicating that the efficiency of pooled sera IQC was comparable to that of a commercial IQC.

As both control materials were analysed for biochemical parameters in the same run, data were interpreted using the within-run Westgard multirule. A rejection rule of 1_{3S}, which is sensitive to random error, was observed for ALP on day 9. Upon cause analysis, it was found that the calibration for ALP was out of limit on that run due to insufficient calibration volume. Corrective action was taken by running calibration with a freshly prepared calibrator. A rejection rule of 2_{2S}, sensitive to systematic error, was observed for electrolytes (Na, K, and Cl) on day 14. An upward trend was also observed with electrolytes due to gradual deterioration of the reagent pack before day 14. Corrective action was taken by replacing it with a new reagent. Another rejection rule of 2_{2S}, sensitive to systematic error, was observed for urea on day 18. Reagent R2 of urea was found to be contaminated due to the mixing of two different lots of reagents. Corrective action was taken by replacing it with fresh reagent.

When the pooled serum was assessed for the stability of routine biochemical parameters over a period of 50 days at -20°C, it was found to be stable for almost all parameters under study except ALT, AST, and bilirubin. The decrease in the concentration of ALT and AST with time could be due to the loss of enzymatic activity as a result of prolonged storage. The decrease in the concentration of bilirubin could be due to its degradation during storage. Although the study observed a statistically significant change in the concentration of these parameters over the 50-day period, the change was found to be clinically insignificant. Similar results have been observed in other studies. For example, Kachhawa K et al., found most of the common biochemical parameters to be stable in serum following 30 days of storage at -20°C [11]. Other studies have also found pooled sera to be better than commercial IQC. Kulkarni S et al., observed variation in the stability of enzymes like AST, ALT, and ALP in the commercial control material after 10 days of storage compared to the pooled serum, where enzymes were stable up to 30 days but the variation was not clinically significant [12].

Khatri R et al., observed that the reliability and validity of test reports were better with pooled sera compared to commercial QC [13]. In this study, pooled sera were run for a period of two months, and it was found to be stable for most of the parameters. Studies have found that the shelf life of pooled sera can be increased by lyophilisation. Jamtsho R prepared homemade lyophilised human serum without a stabiliser, which was stable for atleast nine months when stored at -20°C and seven months at 2-8°C [3]. However, this would require a lyophiliser and may give rise to reconstitution-related errors observed with lyophilised commercial IQC.

Limitation(s)

To prepare a pathological range covering higher values, the addition of analytes would be required. It was difficult to prepare pooled serum IQC covering all pathological ranges at a given point in time.

CONCLUSION(S)

Pooled sera are comparable to commercial IQC in terms of their effectiveness and stability as an IQC.

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