DAILY INTERNAL QUALITY CONTROL IN CLINICAL BIOCHEMISTRY LABORATORY: APPROPRIATE DURATION OF QUALITY CONTROL SERA TESTING FOR DERIVATION OF ITS MEAN AND STANDARD DEVIATION

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ABSTRACT

BACKGROUND

As a part of Internal Quality Assessment, it is a usual practice in Clinical Biochemistry Laboratories to run Internal Quality Control (IQC) sera and check the results for any violation of Westgard rules. A mean and standard deviation (SD) of minimum 20 values of IQC results is taken for plotting Levey-Jennings (LJ) chart, but results of extended period (60 or 90 days) can also be used to calculate mean and SD.

AIMS

- To derive mean and SD of IQC results for a period of 20 days and 90 days.
- To monitor daily IQC results for violation of Westgard rules using mean and SD of 20 days and 90 days.
- To compare frequency of violation of Westgard rules and EQAS performance while using these two means and SDs.

METHODOLOGY

This study was conducted in the Clinical Biochemistry Laboratory of S.S.G. Hospital and Medical College, Vadodara, where two levels of IQC sera are run twice daily. When a new lot of IQC sera was put into use, means and SDs were derived using 20 days (Protocol-I) and 90 days (Protocol-II) results. Both were used for daily monitoring of IQC for 3 months. We compared frequency of violation of Westgard rules (1_{3s}, 2_{2s}, R_{4s}, 10x) and EQAS Standard Deviation Index (SDI) for 10 different biochemical parameters while using these two protocols.

RESULTS

The Westgard rules were violated for a total of 48 times while using Protocol-I as compared to only 5 times while Protocol-II was used. No significant difference was found in EQAS results in terms of SDI.

CONCLUSION

From the current study, it is concluded that for IQC daily monitoring, if mean and SD are derived from longer period (90 days) results of IQC sera, there are fewer incidences of violations of Westgard rules without any compromising effect on EQAS results. Hence, by using more number of values over a longer period, one can reduce unnecessary rejections of run, re-run of IQC and repeated calibration of test parameters, thereby reducing the overall cost of testing and improving the turn-around time (TAT).

KEYWORDS

Internal Quality Control, Mean, Standard Deviation, Westgard Rules, Levey-Jennings Chart.

MESHTERMS

Quality Control.

INTRODUCTION: The principles of quality management, assurance, and control have become the foundation by which clinical laboratories are managed and operated.[1] Quality is defined as conformance with the requirements of users or customers. Quality improvement reduces waste and leads to improved productivity, which in turn reduces costs and provides a competitive advantage.[1]

In the routine operation of clinical laboratories worldwide, the performance of analytical methods is routinely monitored by analysing specimens whose concentrations or activities are known, followed by comparing observed values with known values.[1] Specimens that are analysed for QC purposes are called control materials. They need to be stable, available in aliquots or vials and amenable to analysis periodically over a longtime.[1]

The control material preferably should have the same matrix as the test specimens of interest. In practice, clinical laboratories purchase materials from one of several

companies that manufacture control sera that are supplied as liquid, frozen or lyophilised materials. The later ones are reconstituted by adding water or a specific diluent solution.[1] Two or three different materials should be selected to provide concentrations that monitor performance at different medical decision levels.[1]

As a part of internal quality assessment, it is a usual practice in clinical biochemistry laboratories to run internal quality control (IQC) sera and check the results for any violation of Westgard rules.[2] Following are the most commonly used Westgard rules for interpreting the control data:[2]

- 1_{3s} refers to a control rule where a run is rejected when a single control measurement exceeds mean+3SD limits.
- 2_{2s} refers to the control rule where a run is rejected when two consecutive control measurements exceed the same mean+2SD limit.
- R_{4s} refers to a control rule where a run is rejected when one control measurement in a group exceeds the mean+2SD limit and another exceeds the mean–2SD limit.
- 10x is a rule where a run is rejected when 10 consecutive measurements fall on one side of the mean.

The most common method of comparing the values observed for control materials with their known values is the use of control charts, in which the observed values are plotted versus the time when the observations were made.[1] A mean and standard deviation (SD) of minimum 20 values of IQC results is taken for plotting Levey-Jennings (LJ) chart, but results of extended period (60 or 90 days) can also be used to calculate mean and SD.[2,3]

The IQC procedures are focused on the monitoring of a single laboratory. For comparison of performance of different laboratories, several external quality assurance schemes (EQAS) are available. The two are complementary activities, IQC being necessary for the daily monitoring of precision and accuracy of the analytical method, and external quality assessment being important for maintaining longterm accuracy of the analytical methods.[1]

AIMS:
1. To derive mean and SD of IQC results for a period of 20 days and 90 days.
2. To monitor daily IQC results for violation of Westgard rules using mean and SD of 20 days and 90 days.
3. To compare frequency of violation of Westgard rules and EQAS performance while using these two means and SDs.

METHODOLOGY: The study was carried out at Clinical Biochemistry Laboratory, Medical College and Sir Sayajirao General (S.S.G.) Hospital, Vadodara.

Two levels of IQC sera are run twice daily. When a new lot of IQC sera was put into use, means and SDs were derived using 20 days (PROTOCOL-I) and 90 days (PROTOCOL-II) results. Both were used for daily monitoring of IQC for 3 months (Protocol-I during Jan-Mar, 2015 & Protocol-II during Apr-Jun, 2015).

We compared frequency of violation of Westgard Rules (1_{3s}, 2_{2s}, R_{4s}, 10x) and EQAS standard deviation index (SDI) while using these two protocols.

Various parameters for which the two protocols were compared and the methods employed for their measurement are as follows:
1. Plasma Glucose: Hexokinase method.[4,5]
2. Serum Urea: Glutamate Dehydrogenase (GLDH) – Urease fixed time method.[6,7]
3. Serum Creatinine: Jaffe's method.[8,9]
4. Serum Glutamate Pyruvate Transaminase (Serum ALT): Modified IFCC kinetic method.[10,11]
5. Serum Glutamate Oxaloacetate Transaminase (Serum AST): Modified IFCC kinetic method.[12,13]
6. Serum Alkaline Phosphatase (ALP): IFCC kinetic method.[14]
7. Serum Uric acid: Uricase – Peroxidase (Uricase – PAP) method.[15,16]
8. Serum Cholesterol: Cholesterol Oxidase – Peroxidase method.[17,18]
9. Serum Total Protein: Biuret method.[19]
10. Serum Albumin: Bromocresol green method.[20]

We used commercially available quality control sera for testing various biochemical parameters in this study. No patient specimen was tested in the present study.

RESULTS & ANALYSIS: As defined in material and methods, 10 parameters done in the Clinical Biochemistry Laboratory were included in the study. Their means and SDs were derived according to two protocols as mentioned below:

Protocol I: Means and SDs derived using 20 days results and used during January to March 2015.

Protocol II: Means and SDs derived using 90 days results and used during April to June 2015.

The two protocols were then compared on the basis of violation of Westgard rules and EQAS SDI.

The observations made with respect to various aspects of the study are as follows.

1. **Mean (SD):** Table 1 shows means and standard deviations (SDs) of various parameters obtained while using the two protocols.

Parameters	Mean(SD) Level 1		Mean(SD) Level 2	
	Protocol-I	Protocol-II	Protocol-I	Protocol-II
Plasma Glucose	84(4.5) mg/dL	85(5) mg/dL	275.5(13.75) mg/dL	281(14) mg/dL
Serum Urea	32(1.4) mg/dL	97(2.99) mg/dL	32(2) mg/dL	94(5) mg/dL
Serum Creatinine	2.24(0.11) mg/dL	2.24(0.11) mg/dL	5.17(0.26) mg/dL	5.13(0.265) mg/dL
Serum ALT	28(1.5) U/L	30(2) U/L	91.5(4.75) U/L	94(5) U/L
Serum AST	37(1.86) U/L	35(2) U/L	195(8.05) U/L	183(9.15) U/L
Serum ALP	79(4.99) U/L	80(4) U/L	302(16.49) U/L	305(15) U/L
Serum Cholesterol	244(10.25) mg/dL	244(12) mg/dL	95(4.31) mg/dL	95(5) mg/dL
Serum Uric Acid	4.63(0.18) mg/dL	4.5(0.2) mg/dL	9.06(0.26) mg/dL	8.8(0.4) mg/dL
Serum Total Protein	6.43(0.27) g/dL	6.7(0.3) g/dL	4.05(0.16) g/dL	4.2(0.2) g/dL
Serum Albumin	4.1(0.16) g/dL	4.3(0.2) g/dL	2.65(0.10) g/dL	2.8(0.1) g/dL
Table 1: Mean(SD) of Different Parameters in Protocol I & II				

Table 1 shows that the means obtained using protocols I & II were more or less same while the SDs obtained using protocol II were broad for most of the parameters as compared to those obtained using protocol I.

2. **No. of Violations of Westgard Rules:** Table 2 shows the number of violations of Westgard rules while using the two protocols.

Parameters	1_{3s}		2_{2s}		R_{4s}		$10x$	
	Protocol-I	Protocol-II	Protocol-I	Protocol-II	Protocol-I	Protocol-II	Protocol-I	Protocol-II
Plasma Glucose	2	1	0	0	0	0	1	0
Serum Urea	4	0	0	0	0	0	2	1
Serum Creatinine	0	0	0	0	0	0	0	0
Serum ALT	4	1	6	0	0	0	1	0
Serum AST	2	0	15	1	0	0	1	0
Serum ALP	0	0	0	0	0	0	0	0
Serum Cholesterol	0	0	0	0	0	0	1	0
Serum Uric Acid	0	0	0	0	0	0	2	1
Serum Total Protein	1	0	0	0	0	0	0	0
Serum Albumin	2	0	4	0	0	0	0	0
Table 2: No. of Violations of Westgard Rules								

In present study, the Westgard rules were violated for a total of 48 times while using PROTOCOL-I as compared to only 5 times while PROTOCOL-II was used.

3. **Percentage of Rejections:** Table 3 and figure 1 show the rejections of runs while using the two protocols I & II.

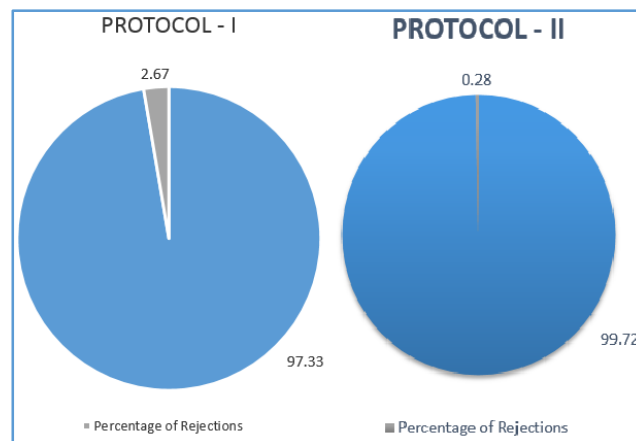|	Protocol I		Protocol II	
	Number	%	Number	%
Total runs	1800	100	1800	100
Runs Rejected	48	2.67	5	0.28
Table 3: Rejection of IQC runs while using Protocol I & II				

Figure 1: Rejection of IQC runs while using Protocol I & II

Table 3 and figure 1 show that the rejection rate was 2.67% (48 out of 1800 runs) for Protocol I while it was only 0.28% (5 out of 1800 runs) for Protocol II).

So, the rate of rejection of IQC run was much less while using the Protocol II.

4. **EQAS SDI:** Table 4 shows the EQAS SDI obtained for different parameters while using Protocol I & II.

PARAMETERS	PROTOCOL-I			PROTOCOL-II		
	January	February	March	April	May	June
Plasma Glucose	-0.19	0.57	0.57	-0.36	0.03	0.58
Serum Urea	-1.00	-0.38	-0.13	-1.16	-1.24	0.01
Serum Creatinine	0.00	0.12	-1.03	0.30	-0.63	1.59
Serum ALT	-0.72	0.27	-0.06	-0.17	-1.44	0.44
Serum AST	-0.87	-0.83	-0.09	-0.85	-1.58	-0.76
Serum ALP	-1.07	-1.19	-1.08	-1.11	-0.99	-0.67
Serum Cholesterol	-0.15	-0.25	-0.39	-0.56	-0.42	0.29
Serum Uric Acid	-0.76	-0.58	-0.49	-1.09	-1.00	0.13
Serum Total Protein	-0.22	-0.52	-0.41	-1.10	-0.86	-0.28
Serum Albumin	-0.03	-0.87	-0.25	-0.69	-0.42	0.53
Table 4: EQAS SDI for different Parameters while using Protocol I & II						

Table 4 shows that the EQAS SDI obtained while using both the protocols were within acceptable limits. So, there is no difference in the EQAS results obtained by using two different protocols.

DISCUSSION: This study was carried out to compare and evaluate two different protocols for derivation of mean and SD of IQC sera. The study was done over a period of 6 months, out of which both the protocols were used for a period of 3 months each.

In this study we found that the rejection rate for IQC runs was 2.67% (48 out of 1800 runs) for Protocol I while it was only 0.28% (5 out of 1800 runs) for Protocol II.

EQAS SDI for all the parameters included in the study was within acceptable limits while using both protocols.

So, for daily IQC monitoring, if mean and SD derived from longer period (90 days), i.e. Protocol II, results of IQC sera are used, there are fewer incidences of violations of Westgard rules without any compromising effect on EQAS results.

The limitation of the present study was that we used the two protocols in different time periods and so, there might be some confounding factors, other than mean and SD, leading to variation of results and thereby rejection of runs.

Further study needs to be carried out to find out which types of error could be deduced and which ones remain unaffected by changing protocols.

CONCLUSION: From the current study, it is concluded that for IQC daily monitoring, if mean and SD are derived from longer period (90 days) results of IQC sera, there are fewer incidences of violations of Westgard rules without any compromising effect on EQAS results. Hence, by using more number of values over a longer period, one can reduce unnecessary rejections of run, re-run of IQC, and repeated calibration of test parameters, thereby reducing the overall cost of testing and improving the turn-around time (TAT).

REFERENCES

1. Klee GG, Westgard JO. Quality management. In: Burtis CA, Ashwood ER, Bruns DE. Teitz Textbook of clinical chemistry and molecular diagnostics. 5th edn. Missouri: Elsevier 2012:163-204.
2. Westgard JO. Internal quality control: planning and implementation strategies. Ann Clin Biochem 2003;40(Pt 6):593–611.
3. Levey S, Jennings ER. The use of control charts in the clinical laboratory. Am J Clin Pathol 1950;20(11):1059-1066.
4. Kunst A, Draeger B, Ziegenhorn J. Metabolites 1: carbohydrates. In: Bergmeyer. Methods of enzymatic analysis. 3rd edn. Vol 6. Verlag Chemie, Weinheim and New York 1984;163-172.
5. Wu AHB, ed. Tietz clinical guide to laboratory tests. 4th edn. Philadelphia: WB Saunders Co 2006;444-451.
6. Tiffany TO, Jansen JM, Burtis CA, et al. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEC fast analyzer. Clin Chem 1972;18(8):829-840.
7. Sampson EJ, Baired MA, Burtis CA, et al. A coupled-enzyme equilibrium method for measuring urea in serum: optimization and evaluation of the AACC study group on urea candidate reference method. Clin Chem 1980;26(7):816-826.
8. Fabiny DL, Ertinghausen G. Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. Clin Chem 1971;17(8):696-700.

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9. Bartels H, Böhmer M. Micro-determination of creatinine. Clin Chim Acta 1971;32(1):81-85.

10. Bergmeyer HU, Hørder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1986;24(7):481-495.

11. ECCLS. Determination of the catalytic activity concentration in serum of L-alanine aminotransferase (EC 2.6.1.2, ALAT). Klin Chem Mitt 1989;20:204-211.

12. Bergmeyer HU, Hørder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC Method for aspartate aminotransferase. J Clin Chem Clin Biochem 1986;24(7):497-510.

13. ECCLS. Determination of the catalytic activity concentration in serum of L-aspartate aminotransferase (EC 2.6.1.1,ASAT). Klin Chem Mitt 1989;20:198-204.

14. Tietz NW, Rinker AD, Shaw LM. IFCC methods for the measurement of catalytic concentration of enzymes part 5. IFCC method for alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1). J Clin Chem Clin Biochem 1983;21(11):731-48.

15. Town MH, Gehm S, Hammer B, Ziegenhom J. A sensitive colorimetric method for the enzymatic determination of uric acid. J Clin Chem Clin Biochem 1985;23:591.

16. Wu AHB, ed. Tietz clinical guide to laboratory tests, 4th edn. St. Louis (MO): Saunders Elsevier 2006;1098-1100.

17. Recommendations for improving cholesterol measurement: a report from the laboratory standardization panel of the national cholesterol education program. NIH Publication No. 90-2964. 1990.

18. Tietz NW, ed. Clinical guide to laboratory tests. 3rd edn. Philadelphia PA: WB Saunders Company 1995;130-131.

19. Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Pathol 1946;10:40-49.

20. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with Bromocresol green. Clin Chim Acta 1971;31(1):87-96.