Low Cost Quality Control Human Serum: Method of preparation, validation of values and its comparison with the Commercial Control Serum

M. A. U. Khan (Department of Pathology, PNS Shifa, Karachi.)
F. A. Khan (Department of Pathology, PNS Shifa, Karachi.)

Introduction

The quality assurance system in clinical chemistry allows for identification of errors and control actions to correct them. Laboratory errors can be classified into pre-analytical, analytical and post-analytical.1 While pre-analytical and post-analytical errors are difficult to identify, the analytical variability (both imprecision and inaccuracy) can be monitored with internal quality control (IQC) programs and external quality assessment (EQA) schemes.2-5 The purpose of IQC is mainly to verify the reliability of laboratory estimates with time. IQC programs are based on the use of control samples which are analyzed in each analytical series.

Freeze-dried (lyophilized) and liquid preparations of commercial sera are available. Many laboratories in Pakistan find it difficult to run IQC because of the non-availability or high cost of commercial quality control sera. It is a common, but un-scientific practice to buy these imported costly materials in small quantity to be used infrequently and to compare the values obtained during analysis with the wide range supplied by the manufacturer. The requirement for quality control sera also include sufficient quality for 1-2 years from the same lot, frequent QC runs as per scientific analytical protocols as well as stability of QC material over the period of intended use. Hence, there was a requirement to prepare liquid quality control serum stabilized with ethylene glycol (which acts as antifreeze agent) using modification of WHO recommended protocol, by a simple process requiring routine laboratory expertise at a considerably less cost.6,7 The home made sera can be obtained from bovine or human source. WHO protocol provides details for preparation of bovine quality control serum. However, based on our previous experience, the bovine serum is difficult to collect, has high concentrations of some constituents e.g. enzymes and haemolysis is frequently encountered during collection and transport. We, therefore, planned a study to validate introduction of home made human serum, obtained from healthy volunteers, for QC of routine clinical chemistry assays and to scientifically analyze its usefulness. We compared it with commercially prepared lyophilized human sera already being used in our laboratory at PNS SHIFA Karachi for seventeen frequently analyzed parameters.

Subjects, Materials and Methods

The liquid human quality control serum stabilized with ethylene glycol was prepared with a modification of the WHO recommended protocol.7-9

After informed consent, four healthy adult donors were phlebotomized in the blood bank at PNS SHIFA in a double bag and the blood was directed towards the bag that did not contain the anticoagulant. After firm clot formation at 37o C, the serum was separated from each bag and was individually screened for HBsAg, Anti-HCV antibodies and Anti-HIV antibodies.10,11

All the four sera were then pooled together in a graduated conical flask and their total volume was measured (580 ml). After mixing thoroughly to ensure homogeneity, the conical flask was placed in a deep freezer (-15 to -20o C) for twenty-four hours to completely freeze the pooled serum.

Next day, the conical flask containing the frozen-pooled serum was placed on a vibration free table at room temperature. The serum was allowed to completely thaw without disturbing until a clear top layer was visible consisting mainly of water or very dilute serum. From this clear top layer, 15% of the total volume (87 ml) was gently pipetted out and discarded. An equivalent volume (87 ml) of ethylene glycol, as preservative and antifreeze agent was added to replace the volume removed. The serum was then mixed thoroughly with ethylene glycol and filtered through nonabsorbent cotton wool to remove any large aggregates.

Two milliliter polystyrene capped tubes were labeled as NC-1 (normal control lot-1) with date. One-milliliter aliquot of the ethylene glycol stabilized QC serum was then pipetted into each tube making a total of 580 aliquots. These aliquots were stored in a

deep freezer (-15 to -20o C) until analyzed.

The liquid control serum stabilized with ethylene glycol was then introduced as a new lot of normal control on Vitalab Selectra II Autoanalyser (Merck Diagnostics) along with the commercial lyophilized human control sera - Level-1 (Normal) (Qualitrol® HSN) and Level-II (abnormal or higher) (Qualitrol® HSP), for comparison of the following seventeen constituents being analyzed routinely on the instrument.

1. Glucose
2. Urea
3. Creatinine
4. Bilirubin

Table 1. Mean, SD, and CVs of seventeen routinely measured analytes in home made human QC serum.

Analytes	Units	Mean	SDs	CVs
Glucose	mmol/l	4.4	0.08	1.8%
Urea	mmol/l	4.2	0.10	2.4%
Creatinine	umol/l	8.8	3.20	3.6%
Bilirubin	umbol/l	7.4	0.40	5.4%
Total Proteins	g/l	70.2	1.50	2.1%
Albumin	g/l	39	0.92	2.4%
Uric Acid	umol/l	268	13	4.9%
Total Calcium	mmol/l	2.2	0.08	3.6%
Inorganic Phosphates	mmol/l	1.3	0.03	2.7%
Magnesium	mmol/l	0.81	0.06	7.4%
Cholesterol	mmol/l	3.4	0.09	2.6%
Triglycerides	mmol/l	1.71	0.09	5.3%
Alanine Aminotransferase (ALT)	U/L	26	1.5	5.8%
Aspartate Aminotransferase (AST)	U/L	20	1.5	5.8%
Alkaline Phosphatase	U/L	213	5	2.4%
Creatinine Phosphokinase (CPK)	U/L	105	5	4.5%
Lactate Dehydrogenase (LDH)	U/L	211	12	5.7%

5. Total Proteins
6. Albumin
7. Uric Acid
8. Total Calcium
9. Inorganic Phosphates
10. Magnesium
11. Cholesterol
12. Triglycerides
13. Alanine Aminotransferase (ALT)
14. Aspartate Aminotransferase (AST)
15. Alkaline Phosphatase
16. Creatinine Phosphokinase (CPK)
17. Lactate Dehydrogenase (LDH)

By running duplicate samples from two vials of the three lots for ten consecutive days we obtained forty values from twenty vials for each analyte. The mean (first target average), standard deviation and co-efficient of variations (CV) for each analyte were then calculated for each lot of the control sera.

Results

Table 1 shows the mean, standard deviation (SD) and co-efficient of variation (CV) of the initial forty values of seventeen analytes in the home made serum.

The comparison of average concentrations (mean) of

Table 2. Comparison of mean concentrations of different analytes between home made QC serum, commercially available Qualitrol HSN and Qualitrol HSP. Reference range of healthy subjects for these analytes in also given.

Analytes	Units	Home-mode QC Serum	Qulitrol®HSN (normal control	Qualitrol® HSP (Higher Control	Reference range	
Glucose	mmol/l	4.4	5.5	13.5	Fasting:	3.3-5.5
					Rondom:	3.3-11.1
Urea	mmol/l	4.2	6.9	23.3		3.3-6.6
Creatinine	umol/l	88	107	368	Male	60-120
					Female	60-106
Bilirubin	umol/l	7.4	22	87		3- 18
Total Proteins	g/l	70.2	51	51.4		65- 85
Albumin	g/l	39	36	32.3		35-50
Uric Acid	umol/l	268	287	574	Male	200-420
					Female	140-340
Total Calcium	mmol/l	2.2	2.2	3.28		2.2-5.5
Inorganic Phosphates	mmol/l	1.3	1.3	2.32	Adult	0.8-1.6
					Children	1.3-2.3
Magnesium	mmol/l	0.81	0.97	1.72		0.7-1.1
Cholesterol	mmol/l	3.4	3.3	3.9		<5.2
Triiglycerides	mmol/l	1.71	1.4	2.28		0.4-2.3
Alanine Amiontransferase(ALT)	U/L	26	41	125	Upto	43
Aspartate Aminotransferase (AST)	U/L	20	40	130	Upto	35
Alkaline Phosphatase	U/L	213	152	525	Adult	56-306
					Children:	Upto645
Creatinine Phosphokinase(CPK)	U/L	110	162	495	Male	Upto165
					Female	Upto 90
Lactate Dheydrogenase(LDH)	U/L	211	308	515		230-460

the seventeen analyzed constituents in the home made serum compared to the commercial sera are given in Table 2. The reference interval used in our laboratory has also been given for enabling to understand the level of control material compared to physiological human concentrations. The results show that our control material was near the middle of the physiological ranges compared to the commercial sera.

Figure shows a bar graph comparing the coefficients of variation (CV) of the seventeen analytes in the home made serum to the two commercial sera (Qualitrol® HSN and Qualitrol® HSP).

For some of the analytes CVs of assays for seventeen routinely measured analytes in human QC serum were mostly less when compared to commercial control serum (Table 2 and Figure).

Discussion

Various types of control material have been used in the laboratory practice. The aqueous solutions of pure substances were used in the clinical laboratories for some time but were soon abandoned due to the fact that clinical specimens with countless substances other than the analyte being measured do not behave like aqueous solution of pure substance in chemical reactions. Some laboratories use the reference sera, used to calibrate instrument as control material. This practice is not at all acceptable as the same material is used to check its value against itself.12 The use of serum at different concentrations of analytes is the most accepted control material in practice.

Commercial control sera are prepared at two or three levels of concentration. These can be used for all routine analyses. The disadvantages of commercial material are vial to vial variation in the concentration of their constituents, no matter how carefully the vials are filled. Reconstitution of material can introduce additional error. They are also very expensive.13

In this study, the preliminary data shows that in the home made serum, the twelve routine chemistry analytes (Glucose, Urea, Creatinine, Bilirubin, Total Proteins, Albumin, Uric Acid, Calcium, Inorganic Phosphates, Magnesium, Cholesterol, Triglycerides) and five enzymes (Alanine Aminotransferase-ALT, Aspartate Aminotransferase-AST, Alkaline Phosphatase, Creatinine Phosphokinase-CPK and Lactate Dehydrogenase-LDH) near the middle of the reference intervals used in our laboratory. This is quite expected as the home made serum was prepared from normal healthy adult donors. Therefore, the ethylene glycol stabilized human serum is a good substitute for the normal commercial serum being used in our laboratory. The narrower coefficients of variation in the home made

serum versus the commercial sera imply a lesser vial to vial variation of the constituent analytes in the home made serum translating into a better potential for error detection in the normal ranges.14 Moreover, the labour involved in the reconstitution of lyophilized sera and potential for introduction of an additional pipetting error during reconstitution process are abolished as the home made serum was appropriately apportioned during the initial preparation into two-milliliter vials adequate for one day usage in the daily analytical runs.

Additional advantages of the home made serum include easy preparation using normal laboratory expertise. It is inexpensive and very cost effective resulting in saving precious foreign exchange for the import of commercial serum.13,15 Being prepared from human serum it resembles and behaves like the clinical specimens during analyses. Further work is in hand whereby the serum is being modified by addition of compounds like glucose, urea, bilirubin, enzymes etc. to elevate the concentration of analytes to medium and high concentrations. This would enable users to carry out quality control checks over a wide analytical range. The only difficulty we came across is the engagement of laboratory personnel in preparation of the material and additional deep freezer space required for its storage.

References

1. Irjala KM, Gronroos PE. Preanalytical and analytical factors affecting laboratory results. Ann Med 1998;30:267-72..
2. Ohman S. Quality control for the clinical chemistry laboratory. Qual Assur 1997;5:79-93.
3. Dastugue B. [Quality control....more and always] Ann Biol Clin (Paris). 2000;58:258.
4. Middle J. External quality assurance. Ann Clin Biochem. 1998;35:549-50.
5. Lalani R, Zafar MN, Khurshid M. Efficacy of internal and external quality control in chemical pathology. J Pak Med Assoc 1988;38:255-9.
6. Lalani R, Molla A, Khurshid M. Efficacy of a home made quality control serum. J Pak Med Assoc 1989;39:317-20.
7. Premachandra P, Wood PL, Hill PG, et al. Preparation and stability of low-cost liquid quality-control serum stabilized with ethanediol. Clin Chem 1987;33:851-2.
8. Browining DM, Hill PG, Vazquez Olozabal DA. Preparation of stabilized liquid quality control serum to be used in clinical chemistry, Geneva, WHO technical document LAB/86 . 4 . 1986, pp 31. (also view at http://www.ifcc.org/divisions/EMD/Documents/Fundamentals-for-EQA.pdf).
9. [N/A] Heuck C. WHO laboratory program. World Health Forum 1998;19:68-70.
10. Simon RG, Langhofer LA Jr, Hendricks EJ. Australia antigen content of commercial quality-control sera. Clin Chem 1973;19:221-2.
11. Sugimoto H, Hashimoto N, Marikawa K, et al. [Commercially available control sera contaminated with anti-HIV antibody, HIV antigen and anti-ATLA antibody] Rinsho Byori. 1988;36:933-8.
12. Bais R, O'Loughlin PD, Philcox JC, et al. Preparation and characterization of a human serum matrix suitable for quality control or reference materials. Pathology 1983;15:15-19.
13. Zafar MN, Syed S. Economy and quality assessment of home made clinical chemistry reagents. J Pak Med Assoc 1992;42:95-7.
14. Hartmann AE. Vial-to-vial variation of a stabilized liquid quality control serum. Am J Clin Pathol 1982;78:345-8.
15. Shtern P, Kratokhvila I, Fridesku B. [Economics of quality control in clinical laboratories] Klin Lab Diagn 1997;7:48-9.

Abstract

Objective: To prepare low-cost quality controls (QC) human serum and scientifically evaluate its advantages/disadvantages when compared with commercially available sera.

Methods:The home made QC serum was prepared as per WHO recommended protocol from four healthy volunteers. It was screened for HIV, HCV and HBV, pooled together and stabilized with ethylene glycol. The initial 40 values were used for calculation of means, SDs and CVs for seventeen routinely measured analytes and results were compared with those of commercially available lyophilized human sera.

Results: The average concentrations of seventeen commonly analyzed constituents were found to be near the middle of the physiological range of healthy subjects and the home made serum could be a good substitute for the commercial serum of normal range. The narrower CVs of the analytes imply a lesser vial to vial variation in the home made sera. Additional advantages include easy preparation, no need for reconstitution and lower cost.

Conclusion: Home made serum is a good substitute for the commercial serum of the normal range especially in developing countries like Pakistan (JPMA 54:375;2004).