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

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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.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 -20°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. Albumin
5. Total Protein
6. Amylase
7. Total Carbonate
8. Inorganic Phosphates
9. Magnesium
10. Chloride
11. Sodium
12. Calcium
13. Inorganic Phosphates
14. Sodium
15. Carbonate
16. Total Phosphates
17. Total Calcium

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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 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.

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 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.
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

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).