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Research Article

ALTERNATIVE METHOD FOR UNINTERRUPTED AND INEXPENSIVE INTERNAL QUALITY CONTROL PROCESS IN CLINICAL BIOCHEMISTRY LABORATORY

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A B S T R A C T

IQC and EQA are the two eyes of the quality assurance in the Clinical laboratory. If one is affected the whole vision of the lab is become dark. To avoid the blind spots in the quality report delivery an uninterrupted QC run is the most essential one. Routine IQC run performance monitoring have greatly increased, due to increasing automation in clinical laboratories. The constant use of commercial QC material is not possible for many laboratories due to non-availability of sample load or not affordable. Here we studied the alternative of IQC using human sera homemade as QC material with anti-freezing agent as a stabilizer. To prepare and validate the inexpensive and stable quality controls (QC) using human sera. The in-house serum based IQC was prepared as per standard protocol using ethylene glycol as anti-freezing agent. The initial 30 values were used for calculation of mean, SD and CV for 21 routinely measured analytes and results were compared with those of commercially available lyophilized human sera. The average concentrations of 21 commonly analyzed constituents were found to be near the middle of the physiological range of healthy subjects and the homemade serum could be one of the good substitutes for the commercial material. The narrower CVs of the analytes imply a lesser vial to vial variation in the home made sera. It is concluded that inexpensive materials can potentially use as QC material.

INTRODUCTION

Total quality management and Quality assurance in clinical chemistry allows for identification of errors and control actions to correct and prevent them. QC plays a major role in monitoring precision, accuracy and reproducibility of results generated and to monitor laboratory performance which is an integral part of any health care laboratory. Laboratory errors categorized as pre-analytical, analytical and post-analytical (Irjala *et al.,* 1998). The purpose of laboratory medicine is to reduce uncertainty is one of the largest contributory factors to the occurrence of diagnostic errors across most specialties in medicine (Bonini *et al.,* 2002). While pre-analytical and post-analytical errors are difficult to identify, the analytical variability (both imprecision and inaccuracy) can be monitored with establishing the theoretical framework and practical routines for single sample-based external quality control (EQC) and batch-oriented routines for internal quality control (IQC) (Ohman *et al.,* 1997, Lalani *et al.,* 1988). IQC material is important for day-to day checking of analytical procedures to ensure patient's reliable laboratory results. However, many medium, small size and non-accredited laboratories are having a difficulty in following the QC procedures. This may be due to lack of awareness, unavailability of sample load to their laboratory and cost of commercial QC materials which is not affordable.

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The total error of a measurement system estimated when measuring control samples is frequently the main emphasis of laboratories despite the fact that the total error only represents in the order of 20% of the diagnostic uncertainty related to laboratory medicine (Lalani *et al*.1989). The Total Analytical Error (TAE) in a clinical laboratory depends upon the maintenance of precision and accuracy, the two watch words for the total reliability of laboratory results. Diagnostics in the clinical chemistry laboratory is a pivotal part of clinical decision making but is not exempt from 'human errors' (IOM, 2000). Many studies have been carried out in the past for the preparation of IQC (Kanagasabapathy, 1996).

Many commercial organizations are supplying freeze-dried (lyophilized) and liquid preparations of commercial sera are available. Many laboratories have their own protocol for the reconstitution ever there are availability of standard guideline. 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 are supplying lyophilized controls with reference values and the guidelines to use such controls are the same that is reconstitution with deionized water and the suggested stability is 7 days when stored at 2-80C (Fraiola *et al*., 1976). The stability beyond 7 days has not yet been established as a result many laboratories discard the reconstituted control leading to frequent use of an expensive material. 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.

In addition, an exact volume of water must be added to lyophilized commercial serum to use the reconstitution, if untrained staff handling the reconstitution process might leads to pipetting error and wastage of control material. This may be happen the laboratories function without any supervision of the technical competent person. Not only this, many interferences a making as choice for the doubt in quality such as quality of water, reconstitution method, pipette used, aliquot storage, time taken for thawing the freezing material, method of thawing (Premachnadra et al., 1987).

Control material prepared should simulate human serum in terms of matrix and concentration of analytes ((Middle et al., 1998; Miller et al., 2003). Serum is the watery portion of blood that remains after coagulation and is free from fibrin. Serum can be obtained by centrifugation of the blood collected into the tube without an anticoagulant. Because the platelets and coagulation factors are activated when blood vessels are punctured, their activation continues in sample tubes, platelets release a small amount of potassium into the serum during the clotting process (Stankovic *et al.,* 2004), therefore the lysis of blood cells (especially the platelets) causes an increase in the concentrations of the platelet components such as potassium (Burtis *et al.,* 2012). It must be sufficiently homogenous, stable and non-infective. There are two main sources of serum QC materials, animal and human. The animal serum is disadvantageous due to matrix effect which requires supplementation with enzymes and other constituents (Browning *et al.,* 1986). Therefore, human serum is suitable for preparing control materials in clinical chemistry. Storage of serum is often necessary in laboratories because of technical issues or to stored serum samples for subsequent verification purposes. The retained sample is preserved for additional/ repeat test on request. Preservation criteria are at 2-8°C for 48hrs (from the time of collection) and disposed at the end of the day by biomedical waste agency (Marjani *et al.,* 2008). But it more stable will stored in -20^{o}C and we were using the disposed or trashed serum sample to prepare a quality control with addition of ethylene glycol as per WHO protocol. Ethylene glycol (IUPAC name: ethane-1,2-diol) is an organic compound with the formula (CH2OH)2. EG is produced from ethylene (ethene), via the intermediate ethylene oxide. Ethylene oxide reacts with water to produce ethylene glycol according to the chemical equation: $C_2H_4O + H_2O \rightarrow HO{-}CH_2CH_2{-}OH$. It is one of the anti-freeze agents. WHO document LAB/81.4 (7) encourages the local production of lyophilized and liquid control serum. The use of ethylene glycol in the preparation of stable liquid serum was described more than three decades ago (Kanagasabapathy *et al.,* 1996) and subsequent studies have shown that such material has useful applications in clinical chemistry. Nevertheless, assess the stability of some clinical chemical analytes in homemade QC from disposed/trashed serum stabilised with ethylene glycol. EG as a preservative to stabilize serum and to use as Internal Quality Control (IQC) was introduced in the early eighties by stabilising it with 30% (V/V) ethylene glycol (Fraiola *et al.,* 1976) Maintenance of day to day accuracy is an indispensable part of any health care clinical laboratory.

EG disrupts hydrogen bonding when dissolved in water. Pure ethylene glycol freezes at about-12 °C (10.4 °F), but when mixed with water, the mixture does not readily crystallize, and therefore the freezing point of the mixture is depressed. Specifically, a mixture of 60% ethylene glycol and 40% water freezes at-45 °C (-49 °F). The antifreeze capabilities of ethylene glycol have made it a component of vitrification **(**anticrystallization) mixtures for low-temperature preservation of serum samples. Mixture of ethylene glycol and water can also be chemically termed as glycol concentrate/compound/ mixture/solution (Rebsdat *et al.,* 2000). 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 (Premachandra *et al*., 1987; Lalani *et al*., 1989).

MATERIALS AND METHODS

The liquid human quality control serum stabilized with ethylene glycol was prepared with a modification of the WHO recommended protocol. (Kenny et al.,1981; Zafar et al., 1992; Henriksen et al. 2004). All the pooled sera were together in a graduated conical flask and their total volume was measured. After mixing thoroughly to ensure homogeneity, the conical flask was placed in a deep freezer (-15 to -20oC) 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 (For Eg 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. Ethylene glycol stabilized QC serum was then pipetted into each tube making a total of 31 aliquots. These aliquots were stored in a deep freezer (-15 to -20oC) until analyzed. Following parameters chosen to perform the daily quality control procedure for 31 days.

1. Alanine aminotransferase | 2. Albumin | 3. Alkaline Phosphatase | 4. Amylase | 5. Aspartate aminotransferase |6. Bilirubin Total | 7. Calcium | 8. Cholsterol | 9. CK-MB | 10. Creatinine | 11. Creatinine Phsophokinase (CPK) |12. Glucose | 13. HDL- Cholesterol | 14. Inorganic Phosphates | 15. Lactate Dehydrogenase | 16. Lipase | 17. Total Protein | 18. Triglycerides | 19. Urea | 20. Uric acid | 21. Gamma Glutamyl transferase.

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

Figures 1 to 4 Shows 21 analytes results for 30 analytical day runs from the liquid human quality control serum stabilized with ethylene glycol prepared by a modification of the WHO recommended protocol, which was stored at 15-20^{0}C as a aliquots These results are obtained with in ± 2 standard deviation.

Table 1 shows the mean, standard deviation (SD) and co-efficient of variation (CV) of the 30 values of 21analytes in the home made serum as IQC material.

Table I shows the mean, SD and CV% for a total of 21 analytes included in this stability study. The %CV results for ethylene glycol stabilised sera control all in acceptable limits.

S.No	Analytes	Methodology	Units	Mean	SD	CV%
1	Alanine aminotransferase (ALT)	IFCC modified UV kinetic	mg/dL	20.79	0.92	4.45
2	Albumin	Bromocresol green	mg/dL	3.45	0.03	1.07
3	Alkaline phosphatase	NPP-AMP-kinetic	mg/dL	92.84	4.05	4.36
4	Amylase	CNP-Triose-CNPG3	mg/dL	55.27	1.53	2.78
5	Aspartate aminotransferase (AST)	IFCC modified UV kinetic	g/dL	26	1.08	4.18
6	Bilirubin total	DPD	mg/dL	0.47	0.08	17.86
7	Calcium	Arsenazo III	mg/dL	8.33	0.21	2.57
8	Cholesterol	Oxidase,esterase,peroxidase	mg/dL	148.92	1.48	0.99
9	CK-MB	NAC activate IFCC	mg/dL	11.91	0.9	7.61
10	Creatinine	Jafee's kinetic	mg/dL	1.53	0.03	2.19
11	Creatinine phosphokinase (CPK)	NAC activate IFCC	mg/dL	123.78	1.75	1.41
12	Glucose	Hexokinase	mg/dL	90.75	1.4	1.54
13	HDL-Cholesterol	Homogenous-GSMBT	mg/dL	31.54	1.14	3.64
14	Inorganic phosphates	Phosphorous-molybdenum blue-deprotenization	U/L	3.43	0.17	5.12
15	Lactate dehydrogenase (LDH)	Pyruvate lactate	U/L	177.1	4.34	2.45
16	Lipase	1,2diglyceridequinone-diamine end point	U/L	38.07	4.83	12.68
17	Total protein	Serum, biuret, kinetic	U/L	6.59	0.11	1.69
18	Triglycerides	End point kinetic	U/L	12840	1.89	1.47
19	Urea	Urease-colorimetric	U/L	32.48	1.06	3.27
20	Uric acid	Uricase-colorimetric	U/L	5.06	0.09	1.85
21	Γ-glutamyl transferase	G-gamma-glutamyl-carboxy-nitroanilide	U/L	50.14	0.77	1.53

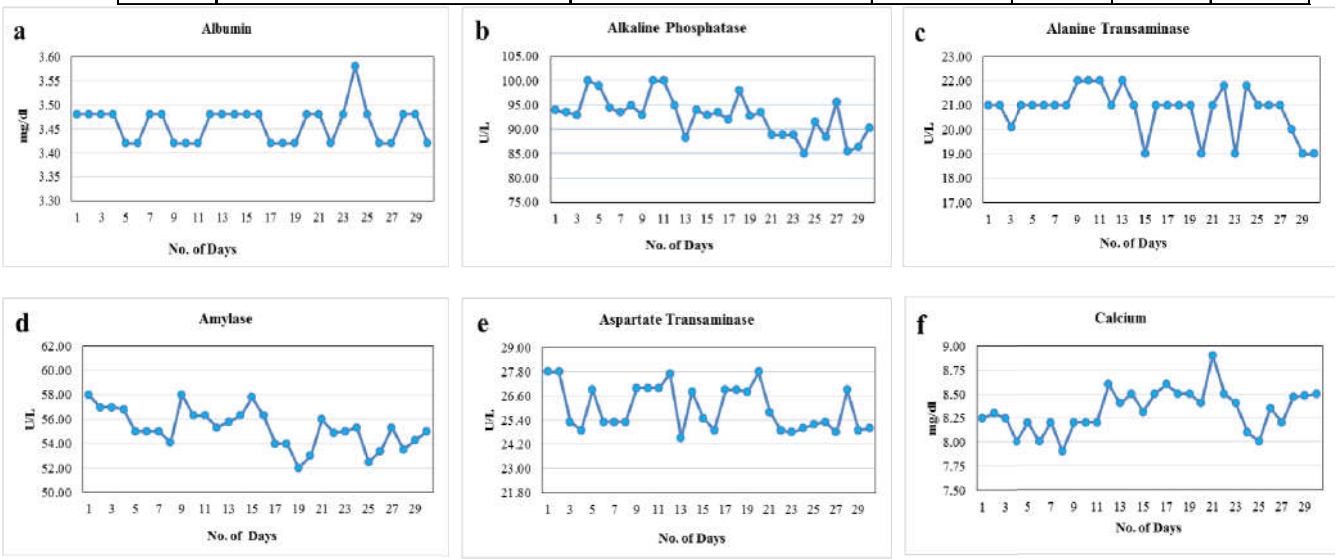

Figure 1 Shows the graph for the analytes of a. Albumin, b. ALP, c. ALT, d. Amylase, e. AST, f. Calcium results for 31 analytical day runs from the liquid human quality control serum stabilized with ethylene glycol which was stored at 15-20 degree Celsius. This results are obtained with in ± 2 standard deviation

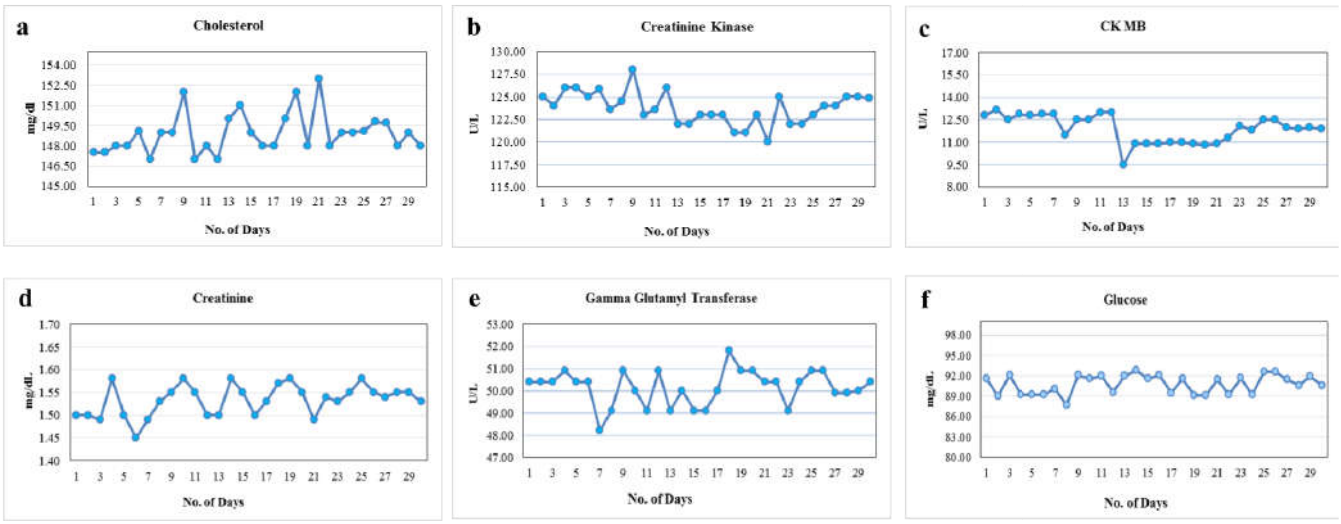

Figure 2 Shows the graph for the analytes of a. Cholesterol, b. CK, c. CK-MB, d. Creatinine, e. GGT, f. Glucose results for 31 analytical day runs from the liquid human quality control serum stabilized with ethylene glycol which was stored at 15-20 degree Celsius. This results are obtained with in ± 2 standard deviation

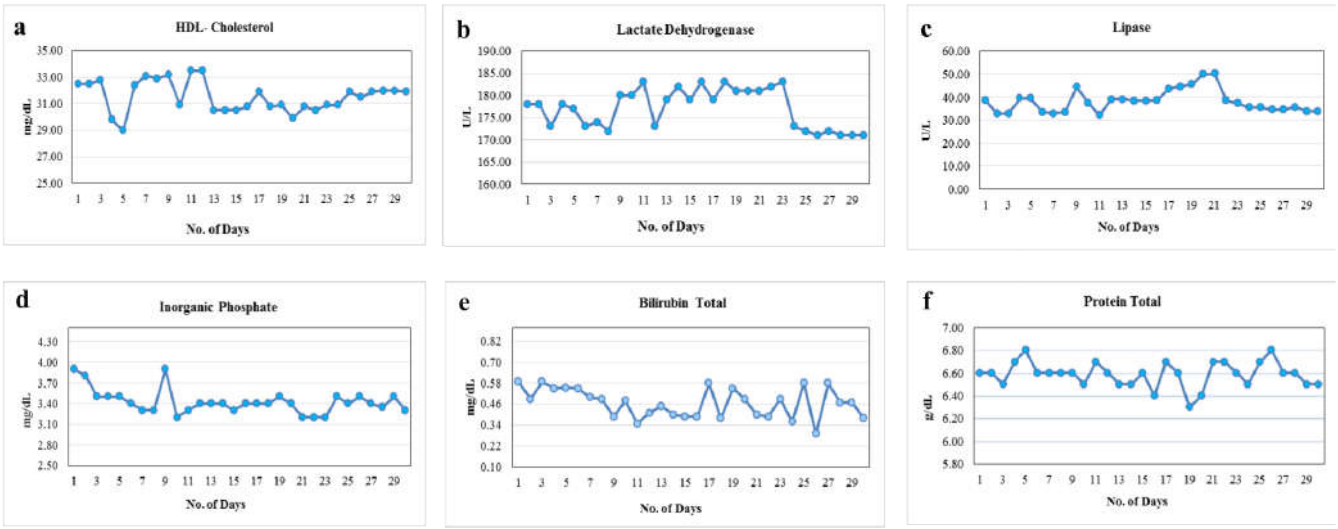

Figure 3 Shows the graph for the analytes of a. HDL, b. LDH, c. Lipase, d. Phosphate, e. Billirubin, f. Protein Total, results for 31 analytical day runs from the liquid human quality control serum stabilized with ethylene glycol which was stored at 15-200 degree Celsius. This results are obtained with in ± 2 standard deviation

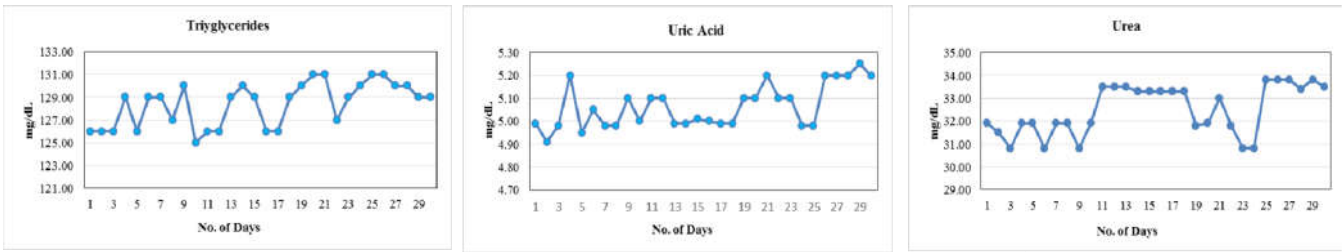

Figure 4 Shows the graph for the analytes of a. Triglycerides, b. Uric Acid, c. Urea, results for 31 analytical day runs from the liquid human quality control serum stabilized with ethylene glycol which was stored at 15-20 degree Celsius. This results are obtained with in ± 2 standard deviation

DISCUSSION

In the midst of growing automation in the laboratory, the requirements for quality control material have significantly increased in order to keep an eye on performance. In general there are three categories of clinical laboratories such as basic laboratory which is managed by the diploma holder with the manual methods, such as using colorimetric methods without controls and calibration, the other is equipped with semi-automated equipment which is performed and managed by the non-medical graduates with paramedical diploma with few years of experience.

Final one is with well-equipped and sophisticated instruments and the laboratory is headed by pathologist, biochemist, and microbiologist with highest degrees with all the following standards like IQC/EQA practices with computerized automated generation of reports. The regular use of commercial control is not economically practicable for many laboratories particularly the basic laboratories, even though the well-equipped laboratories using a commercial good quality control thus to reduce the relative share of total laboratory costs incurred by quality control. On reflection of the World Health Organization Document LAB/81.47which encourages

the local production of quality control material, we tried alternative method of preparation of homemade quality control by the way of pooled human serum stabled by anti-freezing agent of ethylene glycol. Pooled sera using was favored for laboratories by the way storage of serum is often necessary in laboratories because of technical issues or to stored serum samples for subsequent verification purposes. The retained sample is preserved for additional/ repeat test on request. Preservation criteria are at 2°C-8°C for 24 hrs (from the time of collection) and disposed at the end of the day by biomedical waste agency (Marjani *et al.,* 2008). We were using the disposed or trashed serum sample to prepare a quality control with addition of ethylene glycol as per WHO protocol. Which we found was safe and did not have any of the problems.

Thus, homemade quality control sera can easily be prepared in any small laboratory as it does not require complicated skill and it can have it by the owner of using this simple technique. Commercial control sera are prepared at two or three levels of concentration. These can be used for all regular 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 might introduce additional error for untrained staffs and it also the expensive one. This is quite expected as the home made serum was prepared from trashed samples. 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 (Zafar *et al.,* 1992) 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 homemade serum was appropriately apportioned during the initial preparation into eppendorf vials adequate for 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 (WHO-2002, Henriksen *et al.,* 2004). 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.

CONCLUSION

The pooled serum material used to assess the quality is good enough in par with the commercial quality control material. The precession and the accuracy are also commendable. The cost effective preparation of QC material using pooled serum will definitely pave the path for the quality journey to many micro and small size laboratories without any financial burden.

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Conflict of Interest

Nil

References

1. Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in laboratory medicine. Clinical chemistry. 2002; 48(5):691-8.
2. Lalani R, Molla A, Khurshid M. Efficacy of a home made quality control serum. *Journal of Pakistan Medical Association*. 1989; 39:317.
3. Browning DM, Hill PG, Rodr'iguezOlaz'abal DA, World Health Organization. Preparation of stabilized liquid quality control serum to be used in clinical chemistry. [Geneva]: World Health Organization; 1986.
4. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics-e-book. Elsevier Health Sciences; 2012 .
5. Cornes MP, Church S, van Dongen-Lases E, Grankvist K, Guimaraes JT, Ibarz M, Kovalevskaya S, Kristensen GB, Lippi G, Nybo M, Sprongl L. The role of European Federation of Clinical Chemistry and Laboratory Medicine Working Group for Preanalytical Phase in standardization and harmonization of the preanalytical phase in Europe. Annals of clinical biochemistry. 2016;53(5):539-47.
6. Frajola WJ, Maurukas JO. A stable liquid human reference serum. Health laboratory science. 1976;13(1):25-33.
7. Henriksen GM, Pedersen MM, Nørgaard I, Blom M, Blou L, Blaabjerg O, Uldall A. Minimally processed fresh frozen human reference sera: preparation, testing, and application to international external quality assurance. *Scandinavian journal of clinical and laboratory investigation*. 2004;64(4):293-308.
8. Irjala KM, Grönroos PE. Preanalytical and analytical factors affecting laboratory results. Annals of medicine. 1998; 30(3):267-72.
9. Kanagasabapathy AS, Swaminathan S, Selvakumar R. Quality control in clinical biochemistry. *Indian Journal of Clinical Biochemistry*. 1996; 11(1):17-25.
10. Kenny AP, Eaton RH. Practical guidelines for the preparation of quality control sera for use in clinical chemistry. World Health Organization; 1981.
11. Lalani R, Zafar MN, Khurshid M. Efficacy of internal and external quality control in chemical pathology. Journal of Pakistan Medical Association. 1988;38:255.
12. Marjani A. Effect of storage time and temperature on serum analytes. *American Journal of Applied Sciences*. 2008; 5(8):1047-51.
13. Middle JG, Libeer JC, Malakhov V, Penttilä I. Characterisation and evaluation of external quality assessment scheme serum. Clinical chemistry and laboratory medicine.1998; 36(2):119-30.
14. Miller WG. Specimen materials, target values and commutability for external quality assessment (proficiency testing) schemes. Clinica Chimica cta. 2003;327(1-2):25-37.
15. Ohman S. Quality control for the clinical chemistry laboratory. Quality assurance (San Diego, Calif.). 1997;5(2):79-93.
16. Premachandra P, Wood PL, Hill PG, Browning DM, Olazabal DA. Preparation and stability of low-cost

liquid quality-control serum stabilized with ethanediol. Clinical chemistry. 1987; 33(6):851-2.
17. RebsdatS, Mayer D. Ethylene glycol. Ullmann's Encyclopedia of Industrial Chemistry. 2000.
18. Stankovic AK, Smith S. Elevated serum potassium values: the role of preanalytic variables. Pathology Patterns Reviews. 2004;121(suppl_1):S105-12.
19. World Health Organization. Use of anticoagulants in diagnostic laboratory investigations. World Health Organization; 2002.
20. Zafar MN, Syed S. Economy and quality assessment of home made clinical chemistry reagents. *J Pak Med Assoc*. 1992;42:95-7.

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