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Research Article

Stability of Biochemical Analytes in Reconstituted Lyophilised Controls; Deionised Water VS Ethanediol

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ABSTRACT

This paper compares the stability of commercial accuracy controls reconstituted with 15%(V/V) ethanediol with the conventional method of reconstituting with deionsed water. Two vials of the same batch of Beckman Coulter lyophilised control was used for this study. One vial was reconstituted with deionsed water and the other with 15% (V/V) ethanediol. Each lot was aliquoted into several vials (0.3 ml each) and both preparations were stored at -20^{0}C. Assays for a total of 23 biochemical analytes were carried out using each lot on the same day. This exercise was done at regular intervals of time upto one month. Mean and %CV values for each analyte in each preparation was then calculated and the outcome analysed. The interassay %CV obtained for a majority of analytes during one month were less compared to controls reconstituted with deionsed water. These data strongly support that lyophilised controls reconstituted with 15% (V/V) ethanediol shows better stability compared to controls reconstituted with deionsed water. The outcome of this study confirms that commercial controls used in clinical laboratories shows less day to day variations and long term stability when reconstituted with 15% (V/V) ethanediol than with deionised water. Such a procedure will help clinical laboratories to use controls reconstituted with 15%(V/V) ethanediol economically for a longer period of time to save time, labour and cost on commercial controls.

Keywords: Ethanediol, Deionised Water, QC, Mean, %CV, WHO, EQAS.

INTRODUCTION

Quality control (QC) helps clinical laboratories in the identification of variations in laboratory testing and the procedures used to recognize and minimize 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. The use of ethanediol in the preparation of stable liquid serum was described more than three decades ago [(1)] and subsequent studies have shown that such material has useful applications in clinical chemistry. Nevertheless, the potential benefits of such a simply prepared material have not yet been exploited in the less-developed countries. In this study we have assessed the stability of some clinical chemical analytes in commercial serum stabilised with ethanediol. Most serum-based reference calibrators and control materials are lyophilized preparations. The minimum volume dispensed by any commercial preparations are 5 ml and laboratories usually reconstitute with deionised water. As lyophilised materials have no preservative, controls reconstituted with deionised water are to be aliquoted and stored at 2-8^{0}C and the stability, as stated in the leaflet is only 7 days. The stability of the analytes beyond 7 days has not yet been established. This study describes the use of 15% (V/V) ethanediol as solvent to achieve long term stability.

QC techniques are used to monitor day-to-day variations, measured in terms of %CV for precision and %bias for accuracy to assess the reliability of clinical laboratory performance. Application of these techniques will help reduce errors and give both the laboratory and the clinician confidence in the results.[(1)] Ethanediol 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) ethanediol [(2)] and ethanediol-stabilized serum was found to be stable for 1 year when stored at-20^{0}C. Bovine serum stabilized with15%(V/V) ethanediol, have been recommended by World Health Organisation (WHO) to use as IQC for developing countries. [(3)]

Literature reporting stability characteristics of a wide variety of analytes in lyophilized, liquid, and frozen matrices used for control purposes are available. The definition of stability must take into consideration the analytical method employed. Techniques employing multiple measurements over time and using parametric statistics are desirable, but alternate methods have provided useful information and may also be employed depending on the nature of the data base and the frequency of timed measurements.[(4)] The advantages of controls reconstituted with ethanediol are increased savings in labour and time due to frequent reconstitution and use of controls for an extended period of time.[(5)]

Serum prepared using 30%(V/V) ethanediol may be distributed at ambient temperature for External Quality Assessment Programmes(EQAS)(6). Ethanediol did not interfere with the measurement of any of the analytes

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Table 1: Interassay %CV and % bias (Water Vs Ethanediol as diluent

ANALYTE	n	Reference Mean	Water		Ethanediol	
			Mean	%CV	Mean	%CV
GLUCOSE	13	226	215	3.27	233	2.54
UREA	13	160	161	3.93	156	2.86
CREATININE	13	5.26	5.1	5.15	5	4.08
CHOLESTEROL	13	286	304	2.49	296	1.43
TRIGLYCERIDES	13	355	389	6.79	360	2.03
HDL –C	13	-	97	3.88	96	2.72
LDL –C	13	-	138	2.21	133	4.19
T.PROTEIN	13	7.73	7.7	5.7	7.6	3.5
ALBUMIN	13	4.5	4.6	4.13	4.5	2.8
BILIRUBIN-T	13	6.61	6.18	5.92	6.31	3.4
AST	13	146	129	4.7	146	2.69
ALT	13	128	118	4.4	123	3.32
ALP	13	536	500	11.8	537	9.71
GGTP	13	156	137	3.67	155	1.92
CALCIUM	13	13.0	13.5	2.12	13.3	0.57
PHOSPHORUS	13	10.5	11.2	8.6	10	4.95
URIC ACID	13	8.91	10.1	5.7	9.8	0.93
MAGNESIUM	13	4.01	4	3.42	4	4.53
AMYLASE	13	224	210	3.31	226	1.29
CK-T	13	406	370	1.82	410	1.35
SODIUM	13	154	155	3.13	154	1.82
POTASSIUM	13	6.67	6.7	3.17	6.7	1.9
CHLORIDE	13	114	114	3.62	113	1.98

n refers to the number of times analysed at some intervals of time during a period of 1 month.*

tested by using WHO recommended methods. However, WHO did not use manual methods for the evaluation of stability because of the need for the best possible between-batch precision in order to detect changes in the prepared material with time.[3] In a study, chemically preserved pooled human serum for use as internal QC for the determination of Zinc deteriorated over a time, but 15% ethanediol stablished pooled QC was found to be stable for a period of 90 days[7] and in another study, 12 different constituents including AST and ALT, when stablished with 25% ethanediol was found to be stable at room temperature (32^0C) for 9 days with an interassay %CV of 2.3 and such a preparation could be used for EQAS [8].

MATERIAL AND METHODS

Routinely used Beckman Coulter level II lyophilized assayed control was used to carry out this study. 15 ml ethanediol was diluted to 100 ml to get a solution of 15% (V/V). One vial of the control was diluted with 5 ml deionsed water and the other with 15%(V/V) ethanediol. The vials were gently mixed and allowed to stand at room temperature for 15 minutes. These were then mixed gently by inverting. Each lot of the reconstituted control was then aliquoted into 0.3 ml in screw cap vials and stored at -20^0C. Everyday a vial was removed from each set of dilutions and analysed for 23 basic biochemical analytes including enzymes. This exercise was continued for the next one month. The results obtained for all the 23 analytes were tabulated. Mean, SD and %CV were calculated for each analyte in each lot of preparation and the outcome analysed and interpreted.

RESULTS

Table I shows the mean and %CV for a total of 23 analytes included in this stability study. The %CV results for ethanediol stabilised sera are less compared to controls reconstituted with deionsed water. The %CV indicates scatter of results obtained during the study period ie variations in interday results. These data strongly support that lyophilised controls reconstituted with 15% (V/V) ethanediol shows better stability compared to controls reconstituted with deionsed water.

DISCUSSION

Maintenance of day to day accuracy is an indispensable part of any health care clinical laboratory. The Total Analytical Error (TAE) in a clinical laboratory depends upon the maintenance of both precision and accuracy, the two watch words for the total reliability of laboratory results. Many studies have been carried out in the past for the preparation of IQC (1,2,3,6,10). Many commercial organizations are supplying lyophlised controls with reference values and the guidelines to use such controls are the same ie reconstitution with deionised water and the suggested stability is 7 days when stored at $2\text{-}8^0$C. 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. There is

lacunae in the literature about stability of reconstituted materials at temperature $<0^0$C.

CONCLUSION

The outcome of this study confirms that commercial controls used in clinical laboratories when reconstituted with 15% (V/V) ethanediol and stored at -20^0C shows less %CV confirming long term stability. Such a procedure will certainly help clinical laboratories to use controls reconstituted with 15%(V/V) ethanediol for a longer period of time to save time, labour and cost on commercial controls. As most commercial controls comes in 5 ml volume and are expensive for a small clinical laboratory, an economic way of using the control is very important. This paper gives guidelines to use such controls for a longer period of time to save labour and cost of controls.

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REFERENCE

1. A. S. Kanagasabapathy, S. Swaminathan, R. Selvakumar. Quality control in clinical biochemistry.Indian Journal of Clinical Biochemistry; January 1996, Volume 11, Issue 1, pp 17-25.
2. Fraiola WJ, Maurukas J. A stable liquid human reference serum. Health Lab serv 1976; 13: 25-33.
3. Premachandra P, Wood PL, Hill PG, Browning DM, Olazabal DA. Preparation and stability of low-cost liquid quality-control serum stabilized with ethanediol. Clin Chem. ;1987 Jun;33(6):851-2.
4. Noel S. Lawson, Guy T. Haven and George W. Williams. Analyte Stability in Clinical Chemistry Quality Control Materials. 1982 ; Vol. 17, No. 1 , Pages 1-50.
5. Hartmann AE. Vial-to-vial variation of a stabilized liquid quality control serum. Am J Clin Pathol. ; 1982 Sep;78(3):345-8.
6. Lalani R, Molla A, Khurshid M. Efficacy of a home made quality control serum. J Pak med Assoc. ;1989 Dec;39(12):317-20.
7. S.Swaminathan, M.S.Seshadri, Geetha Srikrishna, R.Selvakumar and AS Kanagasabapathy. Ethanediol Stabilised quality control serum for measurement of zinc. Clinica Chimica Acta, ; 215(1993)119-121.
8. A.S. Kanagasabapathy and S.Swamianthan. External quality assurance programme in India: A ten year experience. Progress in Clinical Biochemistry. Proceedings of the 5th Asian-Pacific Congress of Clinical Biochemistry. September 29-Oct 1991, Kobe, Japan.