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Assessment of Bovine Serum as an Internal Quality Control Material in a Clinical Chemistry Laboratory Set-Up at the Komfo Anokye Teaching Hospital in Ghana

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Abstract:-
Background: Commercial lyophilized human sera used by medical laboratories for quality control are very expensive and of limited availability. Bovine serum preserved with ethylene glycol has been known to be a cost-effective and efficient quality control material. The objective of this research is to assess the performance of bovine serum and bovine serum with adjusted analyte concentrations as internal quality control materials.

Materials and Methods: About 400 millilitres ofvenous blood was taken from the jugular vein of fifty adult cattle. The serum was pooled and preserved in a sterile bottle with ethylene glycol. The study was in two phases. In phase one, three aliquots of the bovine serum were analysed weekly for a month. In phase two, the glucose concentration in the stock bovine serum was raised by the addition of a calculated amount of analytical grade glucose. Three aliquots were analysed weekly for a month. The mean, standard deviation (SD), and co-efficient of variation (CV) were calculated for the analytes analysed in the two phases.

Results: The study showed that bovine serum and bovine serum with adjusted analyte concentrations are highly reproducible.
Conclusion: Bovine serum promises to be a suitable supplement to commercial quality control materials that are used for assessment of internal quality control.

Keywords:- *Analytes, Bovine serum, quality control material, medical laboratory.*

I. INTRODUCTION

Quality control may be described as the use of control materials to monitor the accuracy and precision of all the analytical stages of testing (1). Precision is the degree of dispersion in measurements (2). A set of measurements with low variation can be said to be precise (2).Quality control allows medical laboratories to carry out proper diagnosis of patients (3). Quality control materials are usually inserted into the testing process and analysed by being exposed to the same operating conditions as though they were patient samples (4). The main reasons for inserting quality control samples in runs is to determine the reliability of the testing method (5). Performing medical laboratory quality control tests enables medical laboratories to evaluate performance by determining the reliability of the method, equipment, and reagents they routinely use to arrive at precise results that are consistent with World Health Organization (WHO) standards (6). It is practically impossible to have an effective and properly functioning health care system without implementing a good quality control management system (7)**.** Medical laboratory quality control assessments must therefore be treated with all importance to achieve accurate and reliable results in health facilities in Ghana.

According to Cobbina et al, reference human serum preparations are usually used as quality control materials in internal quality control programs for the calibration of laboratory procedures (7). Commercial sera are prepared at two or three levels of concentration and are used for routine testing in the laboratory. These lyophilized human sera have been shown to be stable for one to two years when stored at 2 – 8 ℃ and several years at -20 ℃ or below (8). The disadvantages of commercial quality control materials are vial-to-vial variation and the introduction of errors during reconstitution (9). Commercial human sera are also expensive (10).

Research conducted on the chemical composition of bovine serum has shown that bovine serum has a high degree of similarity to human serum in terms of analyte concentration as compared to the serum of other higher vertebrates (4) (11). It is known to have a high amino acid content, high plasma protein concentration, and a high red blood cell concentration (12)**.** Bovine serum contains about 60-80 g/L of serum protein, 22-36 g/l of albumin, 2.1-9.6 g/L of urea, 132-152 mmol/L of sodium, 3.9-5.8 mmol/L of potassium, 95-110 mmol/L of chloride and 2-18 µmol of bilirubin (11). To allow laboratories to continuously evaluate their performance with a cheaper and more available alternative to commercial quality control serum, the World Health Organisation published a guideline (WHO document LAB/81.4) to encourage the local manufacture of quality control materials by medical laboratories. Although the use of bovine serum presents great potential, very few laboratories have locally produced bovine quality control materials due to reservations they have about the use of bovine serum. Therefore, the scope of the research was to evaluate laboratory performance using bovine serum and bovine serum with adjusted analyte concentrations as internal quality control materials. This study seeks to

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encourage the local manufacture of quality control materials using bovine serum in medical laboratories.

II. MATERIALS AND METHODS

The study involved fifty adult cattle that were tested by a certified veterinary officer at the Kumasi Veterinary Hospital. The cattle were tested to ensure that they were free from communicable diseases and pathogens such as bacteria, virus, and fungi. About 8 ml of venous blood was taken from the jugular vein of the fifty cattle into a serum separator tube using a vacutainer. The total volume of serum that was decanted from the fresh blood collected was about 300 ml. About 250 ml of pure serum remained after centrifugation. The pure serum obtained was carefully poured into a sterile bottle and mixed thoroughly to ensure uniformity. The serum was kept frozen overnight at a temperature of -25℃ to prevent deterioration when left at room temperature. The serum was allowed to thaw undisturbed at room temperature the next day. When completely thawed, about 15% of the top layer of the serum was removed. This 15% of the total serum volume consists mainly of water or very dilute serum (4). The volume of top layer that was removed was replaced with an equal amount of ethylene glycol to preserve the serum sample. The mixture was carefully mixed to ensure that the ethylene glycol was evenly distributed throughout the serum. The serum was kept frozen at -25 ℃. Bovine serum was used because it is cheap, easy to use and readily available.Furthermore, bovine serum was selected as a supplement to commercial human serum because of ethical concerns raised by some scientists about the use of fresh human blood for manufacturing quality control samples other than for transfusion purposes.

The method of stabilization that was used for preserving the serum was stabilization by the addition of chemicals. This method was selected because it produces a cheap source of stable quality control material that is suitable for routine laboratory use. It is a low cost and simple process that requires normal medical laboratory experience. Bovine serum preserved with chemicals requires no reconstitution before use and they have little or no matrix effect on subsequent analytical processes (3). Ethylene glycol was chosen as the preservative for the serum because research has shown that serum containing 15% ethylene glycol is stable for at least a year when stored at -20℃ (13). The analysis was conducted at the Clinical Biochemistry laboratory of the KomfoAnokye Teaching Hospital in Kumasi, Ghana. The analysis was done in two phases. The bovine serum stock was divided into two parts. Twenty Eppendorff tubes were filled with two millilitres of the first part of bovine serum stock. The other part of the serum stock was refrigerated for use in the second phase of the experiment. Three Eppendorff tubes containing bovine serum were tested each week for a period of one month using a Vitreous 5600 Auto-analyzer (Ortho Clinical Diagnostics, United State of America). The Performance Verifier 1 commercial sera with Lot number 27690 from Auto Clinical Diagnostics was used as the reference sera. The analytes that were analysed in the first phase of the study were urea, creatinine, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gama glutamyl transferase (GGT), total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, total cholesterol, triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), glucose, and uric acid.

The glucose concentration in the second portion of the bovine serum stock solution was raised by the addition of analytical grade glucose in the second phase to bring it within the human reference biochemistry range. The glucose concentration was adjusted because it was critically low in phase one and because its concentration could be easily adjusted by the addition of a calculated amount of analytical grade glucose. The resulting serum was then dispensed into twenty Eppendorff tubes. Three tubes containing the bovine serum with adjusted glucose concentration were tested each week for a period of one month to ascertain the stability and reproducibility.

The data was analysed with SPSS 24.0. The mean, standard deviation and co-efficient of variation (CV) of each analyte were determined.

III. RESULTS

In phase one of the study, the co-efficient of variation was 4.32% for urea, 5.80% for creatinine, 3.42% for AST, 16.03% for ALT , 8.12% for ALP, 52.70% for GGT, 3.99% for total protein, 3.15% for albumin, 7.53% for globulin, 8.07% for total bilirubin, 16.77% for direct bilirubin, 13.76% for indirect bilirubin, 3.44% for total cholesterol, 6.67% for triglycerides, 2.72% for HDL Cholesterol, 6.48% for LDL Cholesterol, 14.29% for VLDL cholesterol, 4.37% for glucose and 3.80% for uric acid.

In phase two of the study, the mean and standard deviation (SD) of the glucose concentration that was adjusted and analysed were 5.80 and 0.01 respectively. Also, the co-efficient of variation of the adjusted glucose value was 0.17%.

The recorded mean value of ten analytes including urea, creatinine, ALP, GGT, total protein, globulin, total bilirubin, indirect bilirubin, HDL cholesterol, and LDL cholesterol were within normal human reference biochemistry range. Nine of the bovine analytes were outside of the physiological human reference biochemistry range. Three analytes including AST, ALT, and direct bilirubin were above the human reference biochemistry range and six analytes including albumin, total cholesterol, triglycerides, VLDL cholesterol, glucose, and uric acid, were below the human reference biochemistry range.

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BOVINE BIOCHEMISTRY RESULTS (Phase 1)

	Analyte	Units	Mean	SD	CV
1	Urea	mmol/L	5.55	0.24	4.32%
2	Creatinine	µmol/L	69.08	4.01	5.80%
3	AST	U/L	105.14	3.6	3.42%
4	ALT	U/L	66.32	10.63	16.03%
5	ALP	U/L	85.36	6.93	8.12%
6	GGT	U/L	50.42	26.57	52.70%
7	Total Protein	g/L	65.73	2.62	3.99%
8	Albumin	g/L	34.63	1.09	3.15%
9	Globulin	g/L	31.09	2.34	7.53%
10	Total Bilirubin	µmol/L	10.16	0.82	8.07%
11	Direct Bilirubin	µmol/L	6.38	1.07	16.77%
12	Indirect Bilirubin	µmol/L	3.78	0.52	13.76%
13	Total Cholesterol	mmol/L	2.62	0.09	3.44%
14	Triglycerides	mmol/L	0.15	0.01	6.67%
15	HDL Cholesterol	mmol/L	1.47	0.04	2.72%
16	LDL Cholesterol	mmol/L	1.08	0.07	6.48%
17	VLDL Cholesterol	mmol/L	0.07	0.01	14.29%
18	Glucose	mmol/L	1.83	0.08	4.37%
19	Uric Acid	µmol/L	36.83	1.4	3.80%

Table 1: Bovine Biochemistry results

RAISED GLUCOSE VALUE (Phase 2)

	Analyte	Units	Mean	SD	CV
1	Adjusted Glucose	mmol/L	5.80	0.01	0.17%

Table 2: Bovine Serum with raised glucose concentration

	Analyte	Units	Bovine Serum Mean	Human Reference Range
1	Urea	mmol/L	5.55	2.5 - 8.3
2	Creatinine	µmol/L	69.08	44 - 106
3	AST	U/L	105.14	0.0 - 40
4	ALT	U/L	66.32	0.0 - 41
5	ALP	U/L	85.36	35 - 129
6	GGT	U/L	50.42	12 - 71
7	Total Protein	g/L	65.73	63 - 82
8	Albumin	g/L	34.63	35 - 52
9	Globulin	g/L	31.09	23 - 35
10	Total Bilirubin	µmol/L	10.16	3 - 22
11	Direct Bilirubin	µmol/L	6.38	0 - 5
12	Indirect Bilirubin	µmol/L	3.78	0.0 - 14
13	Total Cholesterol	mmol/L	2.62	3.9 - 5.2
14	Triglycerides	mmol/L	0.15	0.50 - 2.26
15	HDL Cholesterol	mmol/L	1.47	0.90 - 2.20
16	LDL Cholesterol	mmol/L	1.08	0.0 - 3.80
17	VLDL Cholesterol	mmol/L	0.07	0.2 - 1.0
18	Glucose	mmol/L	1.83	3.6 - 6.4
19	Uric Acid	µmol/L	36.83	202 - 417

Table 3: Comparison of mean analyte concentrations of pooled bovine serum with the human reference biochemistry range

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IV. DISCUSSION

The study showed that ten analytes including urea, creatinine, ALP, GGT, total protein, globulin, total bilirubin, indirect bilirubin, HDL cholesterol, and LDL cholesterol were within normal human reference biochemistry range. Three analytes including AST, ALT, and direct bilirubin were above the human reference biochemistry range. This may be indicative of liver damage in some of the cattle that were sampled. Six analytes including albumin, total cholesterol, triglycerides, VLDL cholesterol, glucose, and uric acid were below the human reference biochemistry range. This may be due to malnutrition or impaired hepatic function in some of the cattle that were sampled.

According to Reed et al, a CV of more than 30% may lead to lower precision and a CV below 20% leads to higher precision (14). Thus, we generalized that all the phase one and phase two analyte values, except the values for Gama glutamyl transferase, were highly precise and stable. This implies that there is a high potential for detecting deviations from normal reference ranges should bovine serum be used for routine testing.

The analytes whose concentrations were outside of the human reference range can be modified to fall within the human reference range as was demonstrated by the adjustment of the glucose concentration in phase two. The modification of the concentration of most analytes can easily be carried out by hand either by the addition of more analyte or by dilution (4).

Bovine serum preserved with ethylene glycol promises to provide laboratories with a cost effective and efficient material for routine analysis. When used, operational cost will be reduced because the serum can be prepared on a low budget. Moreover, no professional expertise is required to formulate the material as normal laboratory experience would suffice. No labour will be required for reconstituting the serum as the material is already in the liquid form. Also, the ability to modify the concentration of bovine serum analytes greatly increases its potential for use as a quality control material for a wide analytical range on a large scale.

V. CONCLUSION

The study demonstrated that bovine serum preserved with ethylene glycol promises to be a suitable supplement to commercial quality control materials used for evaluating laboratory performance in internal quality control assessment. The study also showed that bovine serum with adjusted analyte levels is reproducible and promises to be good for routine testing. Bovine serum shows the potential of being able to boost laboratory performance and cut cost without compromising the quality of the tests conducted. Since the study was conducted in a single laboratory using a single auto-analyser, more research needs to be done to determine the applicability of bovine serum for the evaluation of laboratory performance in external quality control assessment. Also, since this study was conducted using only bovine serum at the normal levels of analyte concentration, more research will be needed to determine its performance at low and high levels of analyte concentrations.

VI. LIST OF ABBREVIATIONS

SD	Standard deviation
CV	Co-efficient of variation
WHO	World Health Organization
EQA	External quality assessment
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
GGT	Gama glutamyl transferase
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
VLDL	Very Low-Density Lipoprotein

DECLARATIONS

- **Conflict of Interest:** The authors declare that they have no competing interests.
- **Funding:** The authors received no funding
- **Ethical approval:** Ethical approval was sought from the Ethics Committee of the KomfoAnokye Teaching Hospital in Kumasi, Ghana.
- **Guarantor:** Eliezer Togbe, PhD

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