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Assessment of bovine plasma and serum sample stability at different storage conditions with concentration variations of biochemical analytes

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ABSTRACT

Aim: This study aimed to evaluate the long-term stability of cattle plasma and serum, measuring changes in the concentration of some biochemical analytes under different storage temperatures and time conditions and assessing the potential clinical impact of these changes.

Methods: A total of 7 biochemical analytes in the serum and plasma of seventy-four adult cattle were examined following storage. After determining the baseline measurements (D0), the serum and plasma of each cattle were aliquoted and stored at 4°C and −20°C for 15, 45, 75, 105, 135, 165, and 180 days and then analyzed for stability. The results were compared with the initial analysis measurements obtained from fresh samples. The mean concentrations of biochemical analytes in different storage conditions were compared to the initial concentrations (D0) using a paired *t*-test and reference values to evaluate the potential clinical impact.

Results: The glucose, creatinine, and cholesterol were stable under all conditions. Glucose and aspartate aminotransferase (AST) concentration in serum versus plasma showed significant differences ($p < 0.05$). Uric acid had a clinically significant loss of stability under all conditions ($p < 0.05$). The instability for total bilirubin (TB) at D180 was stored at 4°C and −20°C ($p < 0.05$) in serum and plasma, but the potential clinical impact was statistically significant in only serum samples. The instability for total protein (TP) at D45, D135, D165, D180, and D15, D75, D105, D135, D180 stored at 4°C and −20°C ($p < 0.05$) in the serum samples, respectively. The potential clinical impact was observed at D165 to D180 stored at 4°C and D180 stored at −20°C in the serum sample. The means of AST were a significant ($p < 0.05$) variation in storage at D180 in (−20°C) serum samples without clinical impact and at D165 and D180 in (4°C) plasma samples with clinical impact.

Conclusion: Serum samples were comparatively better than plasma samples for most biochemical analytes. Storing samples at −20°C is a better way to preserve TB, TP, and AST. Therefore, the samples had to be kept in separate aliquots for re-analysis as the stability of the analytes decreased if thrown and stored again. However, this study will provide reference results for interpreting clinical diagnostic reports and determining the temperature and time length for storing laboratory samples.

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Introduction

In Bangladesh, current biochemical testing through an automated platform base has become very important and popular in the accurate diagnosis of animal diseases. Clinical laboratory results are the basis for 70% of all medical decisions and 70% of a

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patient's medical record [1]. Due to a lack of clinical laboratories, samples are transported from distant peripheral veterinary hospitals, clinics, or farms. Sometimes samples take longer to reach the central clinical laboratory due to transportation issues. The plasma and serum stabilities of prescribed analytes become strongly dependent on transport times and storage conditions [2]. The prolonged storage of plasma and serum causes significant variations in the concentrations of various biochemical analytes, as demonstrated by potassium (K), lactate dehydrogenase (LDH), or phosphorus (PHOS) [3]. The stability of the analytes in serum and plasma is crucial for clinical laboratories, especially if there is a delay in their processing or they need to be preserved for future investigation [4]. The stability of analytes during sample storage is a widespread problem (approximately 70% of the total errors) in clinical laboratories because variations due to lack or reduced stability reflect the results of laboratory tests employed for making clinical or therapeutic patient decisions [5,6]. The use and reuse of this primary sample resulted in false concentration measurements due to uncontrolled storage conditions [7]. On other occasions, the samples are transported from more or less remote locations, and they are kept in a variety of storage conditions until the time of the analysis, which frequently does not match quality standards [8]. All of this results in the analysis method undergoing changes or degradation processes, which can add up gradually and possibly change the values of the initial sample [9]. The ability of a sample or specimen material to retain its properties over time is referred to as the stability of a biochemistry analyte. As a result, loss of stability is calculated because of property variation and time at particular storage conditions [10]. The stability of an analyte depends on different factors such as cellular metabolism [11], exposure to light [12], tube storage position and spinning [10], adsorption [13], temperature [14,15], contact with air, diffusion, and evaporation [16]. The temperature has a pivotal role as a catalyst in the chemical procedures that contribute to stability loss [17]. The stability of some analytes can also be influenced by storage temperature in addition to storage time [18]. Contact with cells results in a material exchange that does not necessarily involve cell extinction. Electrolytes, substrates of intermediate metabolism, and, to a lesser extent, complex compounds are the substances most affected by contact with cells. In plasma rather than serum, the presence of cellular material remains after centrifugation, and separation from cells is more common, and it depends on the separation and centrifugation method used [19]. On the other hand, locally relevant reference ranges are essential in the clinical diagnosis of diseases and management of affected bovine, allowing for the screening of participants to identify the affected bovine on the farm. There is a lack of an established panel of locally relevant standard reference parameters for biochemical tests in the clinical diagnostic laboratory of large ruminants in Bangladesh. However, this study will provide reference results for clinical diagnostic report interpretation and temperature with time length for the sample storage in laboratories. Comparative large sample sizes, long storage time with a specific time interval to re-analysis, and well-representative analysis of the data are the main strengths of the study. Although several studies have been done about animal plasma and serum stability, little attention has been paid to cattle plasma and serum stability. Therefore, the goal of this study was to evaluate the long-term stability of cattle plasma and serum under different storage conditions, examining the effects of these conditions on the concentration of some biochemical analytes.

Materials and Methods

Ethics approval and consent to participate

Permission was obtained from the farm owner before collecting blood samples from the cattle. All animal studies were conducted following the experimental practices and approved by the ethics committee of Chattogram Veterinary and Animal Sciences University, Bangladesh, with the decision number CVASU/Dir(R&E) EC/2023/ 551-1-10. The clinical examination and blood sample collection from the cattle should be conducted in a manner that minimizes any conditions that could affect animal welfare, in accordance with the standard guidelines and procedures set by the WVU IACUC [20].

Sample collection from cattle

Blood samples were collected from 74 healthy non-pregnant crossbreeds (Holstein Friesian×Local) cattle of Green Harvest Agro Farm, Chattogram, Bangladesh, and each was within 2 years to 2 years 5 months old. These cattle have not been affected by any infectious or chronic disease in the previous history, and no antibiotics or any other drugs have been administered for any acute illness. Cattle were fed a balanced ration containing 40% concentrate and 60% roughage. Cattle were

reared in the loose housing system without any stress. The samples were collected in the morning from 6:30 am to 8:00 am. Before sample collection, all cattle were measured for physiological parameters, including rectal temperature, heart rate, and respiration rate. The average values were averaged at 101.5°F, 63.2 beats/minute, and 26.8 breaths/minute, respectively. Blood was collected from the cattle's caudal vein (tail vein) using a 50 ml syringe with an 18 G needle, with a total volume of 40 ml. The 40 ml of blood collected from each cattle was divided into two (20 ml aliquots). One 20 ml aliquot was collected in an evacuated tube with NaEDTA for plasma collection, and the other 20 ml aliquot was collected in a Falcon tube without anticoagulant for serum collection. The 74 NaEDTA tubes were centrifuged at 2,000 g for 10 minutes for plasma collection, and another 74 Falcon tubes were placed vertically in a rack for 30 minutes at room temperature for serum collection. The plasma and serum were separated from the whole blood within one and a half hours of blood collection. Therefore, there were no delays in transportation and no effect on environmental temperature.

Biochemical analysis

Ten milliliters of plasma and equal amounts of serum were collected from each sample. Then, plasma and serum samples were placed in eight aliquots in sterile plastic tubes with lids.

In the primary Falcon-serum and NaEDTA-plasma tubes (D0), seven clinical chemistry analytes were quantified in duplicate: glucose, total bilirubin (TB), creatinine, uric acid (UC), total protein (TP), cholesterol (Chol), and aspartate aminotransferase (AST). These analytes were measured using a Humalyzer 3,000 biochemical semi-autoanalyzer from Roche Diagnostics (Germany) in the Biochemistry Laboratory at the Department of Physiology, Biochemistry and Pharmacology, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

The equipment had previously been calibrated and controlled by our own analytical quality assurance procedure, which was established by the laboratory's management system in NetLab (Synlab Ecuador). Analyte concentrations were determined using respective reagents against each biochemical analyte. The determination of biochemical analyte concentrations was performed using reagents from the following companies: Glucose (Bioscience Medical S.L., C/Velazquez 1267®, Madrid, Spain), TP and Chol (AMEDA Labordiagnostik GmbH®, Krenngasse 12, 8010 Graz, Austria), TB, creatinine, and AST (Randox Laboratories Limited®, Crumlin, BT29 4QY, United Kingdom), and UC (HUMAN Gesellschaft für Biochemica und Diagnostica mbH®, Max-Planck-Ring 21, 65205 Wiesbaden, Germany). The analysis followed the standard procedures for each analyte as outlined in the respective company manuals.

Seven aliquots of serum and seven aliquots of plasma were stored at 4°C and seven aliquots of serum and seven aliquots of plasma were stored at –20°C. Seven aliquots per cattle were stored for 15 days (D15), 45 days (D45), 75 days (D75), 105 days (D105), 135 days (D135), 165 days (D165), and 180 days (D180). To avoid changes induced by environmental factors such as evaporation or light exposure, all aliquots were maintained in the dark in tightly sealed containers and placed vertically. All stored aliquots were strongly maintained at the respective temperatures and were continuously checked by the digital thermometer. The aliquots were thawed at room temperature (24°C) for 45 minutes before the experimental stability tests, and the seven biochemical parameters in serum and plasma were then measured simultaneously, using the same procedures and the same circumstances as the reference measurement (D0).

Statistical Analysis

After analysis of plasma and serum samples, the values of each analyte were compiled into Microsoft Excel Professional 2020 (Microsoft Corporation, USA). After coding and re-coding, data were checked for integrity and consistency and then entered STATA 13 (Stata Crop, 4905, Lakeway Drive, College Station, Texas 77845, USA) for statistical analysis. The median and IQR (inter-quartile range) were calculated for each analyte. The % of concentration change from baseline (% change) was calculated for each analyte and each storage time using the following formula:

$$\%\text{change} = \frac{D_X - D_0}{D_0} \times 100$$

where D_0 represents the initial median value, defined as the analyte concentration for the first tube (kept ~2 hours) measured immediately after collection, and D_x represents the median value of the measured values at D15, D45, D75, D105, D135, D165, and D180.

Finally, the reference change value (RCV) was calculated using the following formula:

$$RCV = Z \times \sqrt{2} \times \sqrt{(CVa^2 + CVi^2)}$$

where Z is the Z-score corresponding to the desired confidence level, CVa is the analytical variation coefficient, and CVi is the intra-individual variation coefficient.

The RCV is a measure of the change in a biomarker that is significant enough to indicate a potential clinical impact. It is equivalent to the sum of the square root of 2, the statistic $Z = 1.9$ (obtained from the normal distribution table, with a 95% confidence interval), and the sum of CVi of the analyte being measured and CVa of the laboratory's coefficient of analytical variation. We assume that there was no potential clinical effect of storage conditions on analyte concentration if the % change is less than the calculated RCV. However, if the % change is greater than the calculated RCV, this mirrors the potential clinical impact, which in our study refers to the instability of the analyte evaluated.

The difference between serum and NaEDTA-plasma was evaluated by paired t-test on D0. The probability value of $p \leq 0.05$ was considered as a cutoff in evaluating the significant difference.

Results

The present study revealed instability for glucose at D180 and D75, D105, D135, D165, and D180 stored at 4°C and −-20°C ($p < 0.05$), respectively, but the potential clinical impact was insignificant (Table 1). The instability for TB at D180 was stored at 4°C and −20°C ($p < 0.05$), but the potential clinical impact was statistically significant in only serum samples (Table 2). The instability for UC at D15 to D180 was stored at 4°C and −20°C ($p < 0.05$). The potential clinical impact significance was observed at D75 to D180 stores at 4°C and D15 to D180 stores at −20°C in the serum sample, but all the stored plasma samples were statistically significant (Table 4). The instability for TP at D45, D135, D165, D180, and D15, D75, D105, D135, D180 stored at 4°C and −20°C ($p < 0.05$) in the serum samples, respectively. The potential clinical impact was observed at D165 to D180 stored at 4°C and D180 stored at −20°C in the serum sample (Table 5). The instability for AST at D45, D135, D165, D180, and D15, D165 was stored at 4°C and −20°C ($p < 0.05$), respectively, but the potential clinical impact was observed at D165 and D180 in the plasma sample (Table 7). Glucose and AST concentration in serum versus plasma showed significant differences ($p < 0.05$) (Tables 1 and 7).

Discussion

In the modern era, Large ruminant medicine relies on diagnostic tests, such as hematologic analyses, to assess the health status of individuals. Knowledge regarding Large Ruminant Pathology has progressed slowly in Bangladesh because of the difficulty developing standard intervals for reference values in the regional Large ruminant blood biochemical profile.

Despite the similarities between serum and plasma, the processing of the two samples is different. Plasma was separated from whole blood by centrifugation within 10 minutes. The serum was separated from the whole blood by allowing the blood to clot within half an hour. Since the fluid component of blood is in contact with blood cells for a longer period for serum compared with plasma, the effects induced by metabolic changes in serum may be greater. The National Committee for Clinical Laboratory Standards guidelines for mammalian blood samples recommend the separation of the plasma or serum within a maximum time interval of 2 hours from the time of blood collection [21].

This study's results showed that the analytes' concentrations in serum and plasma were not equivalent for all biochemical analytes. The result was supported by previous researchers who reported that plasma and serum cannot be used as substitutes for one another [22,23]. The serum is qualitatively diverse from plasma in that the bulk of fibrinogen has been evacuated in serum by transformation into a fibrin clot in conjunction with red blood cells, white blood cells, and platelets that have been either physically bound to the fibrin matrix and/or enacted to make aggregates [24]. The previous study further reported that the significant differences demonstrated between serum and plasma were because of changes occurring in the serum sample resulting from long-time contact with the red blood cells [25]. On the other hand, plasma still contains fibrinogen since clot formation has been avoided by the presence of anticoagulants during blood collection. The reciprocal differences between plasma and serum that provided significant errors can be presented in either the analytical or the interpretative phases [22]. Contrastingly, the analyte concentrations in serum and plasma were equivalent [4]. The World Health Organization further adds that the heparin plasma samples were not recommended for all analytical methods that involve glucose and others [4]. Most

Table 1. Glucose concentration in serum and plasma samples at different preservation times (Day) and temperatures.

		Serum						Plasma					
Analytes	**Stability**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**
Glucose (4°C) (mg/dl)	D0	51.5	40.5–54.5	-	-	-	-	57.7	56.3–60.4	-	-	-	-
	D15	52.1	41.3–53.6	1.17	0.7748	-	No	53.6	53.3–63.1	–7.11	0.3753	-	No
	D45	56.5	50.1–58.5	9.70	0.0959	-	No	60.9	58.1–61.1	5.54	0.2271	-	No
	D75	44.4	41.8–48.3	–14.05	0.3733	-	No	50.6	44.5–51.4	–12.30	0.0118	-	No
	D105	56.3	48.5–57.5	9.32	0.5286	87.13	No	66.2	58.1–80.3	14.73	0.6603	79.43	No
	D135	57.0	43.9–70.9	10.68	0.2064	-	No	68.1	67.2–75.1	18.02	0.2394	-	No
	D165	60.9	49.1–65.2	18.26	0.2582	-	No	50.2	28.8–59.5	–13.00	0.1257	-	No
	D180	10.5	5.5–17.1	–79.61	0.0041	-	No	22.0	10.3–30.0	–61.87	0.0073	-	No
Glucose (−20°C) (mg/dl)	D0	52.2	49.6–58.8	-	-	-	-	59.5	57.8–60.6	-	-	-	-
	D15	57.7	49.3–62.6	10.53	0.367	-	No	59.1	57.4–63.8	–0.67	0.9267	-	No
	D45	54.8	48.9–48.9	4.99	0.3559	-	No	59.6	57.2–66.7	0.17	0.8556	-	No
	D75	42.8	39.6–48.7	–18.00	0.0449	-	No	49.1	45.2–56.5	–17.48	0.0325	-	No
	D105	66.9	56.6–72.4	28.17	0.0272	75.67	No	69.2	66.1–76.6	16.30	0.0026	43.11	No
	D135	70.3	52.2–71.3	34.67	0.0427	-	No	72.8	70.2–78.6	22.35	0.0491	-	No
	D165	50.7	40.3–54.3	–2.87	0.0322	-	No	55.4	50.9–57.3	–6.89	0.0116	-	No
	D180	28.8	13.5–31.5	–44.83	0.0142	-	No	45.1	42.1–50.4	–24.20	0.0182	-	No
p-value serum versus plasma								0.0025					

Table 2. Total bilirubin concentration in serum and plasma samples at different preservation times (Day) and temperatures.

		Serum						Plasma					
Analytes	**Stability**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**
Total Bilirubin (4°C) (mg/dl)	D0	0.1	0.1–0.2	-	-	-	-	0.1	0.1–0.2	-	-	-	-
	D15	0.1	01–0.1	0.00	0.1778	-	No	0.1	01–0.1	0.00	0.0705	-	No
	D45	0.1	0.1–0.2	0.00	0.6213	-	No	0.1	0.1–0.2	0.00	0.6213	-	No
	D75	0.1	0.1–0.3	0.00	0.4733	-	No	0.2	0.1–0.3	100.00	0.242	-	No
	D105	0.2	0.1–0.3	100.00	0.208	227.1	No	0.1	0.1–0.2	0.00	0.6213	217.8	No
	D135	0.2	0.1–0.2	100.00	0.3739	-	No	0.1	0.1–0.2	0.00	0.6213	-	No
	D165	0.1	01–0.1	0.00	0.1778	-	No	0.2	0.1–1.1	100.00	0.2397	-	No
	D180	1.2	0.7–1.6	1100.00	0.0415	-	Yes	0.1	0.0–0.1	0.00	0.0161	-	No
Total Bilirubin (–20°C) (mg/dl)	D0	0.1	0.1–0.2	-	-	-	-	0.1	01–0.1	-	-	-	-
	D15	0.1	0.0–0.2	0.00	0.3739	-	No	0.1	01–0.1	0.00	0.3739	-	No
	D45	0.1	0.1–0.2	0.00	1.000	-	No	0.1	01–0.2	0.00	0.5529	-	No
	D75	0.1	0.1–0.1	0.00	0.1778	-	No	0.1	01–0.1	0.00	0.3739	-	No
	D105	0.1	0.1–0.2	0.00	0.3739	216.1	No	0.1	0.1–0.2	0.00	0.6213	204.9	No
	D135	0.1	0.0–0.1	0.00	0.208	-	No	0.1	01–0.1	0.00	0.6213	-	No
	D165	0.1	0.1–0.1	0.00	0.3739	-	No	0.1	01–0.1	0.00	0.6213	-	No
	D180	0.4	0.3–1.2	300.00	0.0452	-	Yes	0.0	0.0–0.1	–100.00	0.0161	-	No
p-value serum versus plasma								0.1649					

Table 3. Creatinine concentration in serum and plasma samples at different preservation times (Day) and temperatures.

		Serum						Plasma					
Analytes	**Stability**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**
Creatinine (4°C) (mg/dl)	D0	1.1	1.1–1.3	-	-	-	-	1.1	1.1–1.2	0.00	-	-	-
	D15	1.2	1.2–1.4	9.09	0.3359	-	No	1.1	1.1–1.3	0.00	0.0705	-	No
	D45	1.3	1.2–1.3	18.19	0.2109	-	No	1.1	1.1–1.1	0.00	0.6213	-	No
	D75	1.3	1.2–1.3	18.19	0.240	-	No	1.2	1.1–1.4	9.09	0.0341	-	No
	D105	1.2	1.2–1.3	9.09	0.3079	132.87	No	1.0	1.0–1.2	–9.09	0.8541	43.67	No
	D135	1.2	1.2–1.3	9.09	0.2408	-	No	1.2	1.1–1.4	9.09	0.208	-	No
	D165	1.2	1.1–1.2	9.09	0.4411	-	No	0.9	0.9–1.2	–18.18	0.1087	-	No
	D180	0.8	0.4–1.0	–27.27	0.2606	-	No	1.1	1.1–1.1	0.00	0.8276	-	No
Creatinine (–20°C) (mg/dl)	D0	1.0	1.0–1.2	-	-	-	-	1.0	0.9–1.0	-	-	-	-
	D15	1.2	1.2–1.3	20.00	0.0516	-	No	1.2	1.1–1.2	20.00	0.0008	-	No
	D45	1.2	1.1–1.2	20.00	0.242	-	No	1.1	1.1–1.2	10.00	0.0046	-	No
	D75	1.2	1.0–1.3	20.00	0.8276	-	No	1.2	1.2–1.3	20.00	0.0006	-	No
	D105	1.0	1.0–1.1	0.00	0.6885	55.66	No	0.9	0.8–1.0	–10.00	0.0161	34.24	No
	D135	1.2	1.2–1.3	20.00	0.1087	-	No	1.1	1.0–1.1	10.00	0.0161	-	No
	D165	1.2	1.2–1.3	20.00	0.0349	-	No	1.0	1.0–1.1	0.00	0.4263	-	No
	D180	1	0.9–1.4	0.00	0.6072	-	No	1.3	1.2–1.4	30.00	0.0132	-	No
p-value serum versus plasma								0.2396					

Table 4. Uric acid concentration in serum and plasma samples at different preservation times (Day) and temperatures.

		Serum						Plasma					
Analytes	**Stability**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**
Uric acid (4°C) (mg/dl)	D0	1.17	0.94–1.17	-	-	-	-	0.44	0.33–0.47	-	-	-	-
	D15	2.00	1.68–2.27	70.94	0.001	-	No	1.65	1.37–2.51	275.00	0.0099	-	Yes
	D45	1.93	1.52–2.17	64.96	0.0073	-	No	1.91	1.63–2.65	334.09	0.0541	-	Yes
	D75	3.33	3.21–3.73	184.62	0.0019	-	Yes	4.45	4.13–4.92	911.36	0.0011	-	Yes
	D105	3.94	3.58–5.07	236.75	0.0034	136.49	Yes	4.68	4.56–4.68	963.64	0.0007	112.99	Yes
	D135	3.63	3.61–3.85	210.26	0.0138	-	Yes	3.52	3.49–3.55	700.00	0.0036	-	Yes
	D165	4.23	3.37–4.62	261.54	0.0048	-	Yes	4.84	4.54–4.93	1000.00	0.0024	-	Yes
	D180	3.14	2.32–3.38	168.38	0.0435	-	Yes	3.12	3.05–3.74	609.09	0.0018	-	Yes
Uric acid (−20°C) (mg/dl)	D0	0.89	0.79–1.0	-	-	-	-	0.30	0.27–0.47	-	-	-	-
	D15	3.81	2.14–4.43	328.09	0.0429	-	Yes	2.01	1.41–2.69	570.00	0.004	-	Yes
	D45	1.81	1.53–2.03	103.37	0.0272	-	Yes	1.50	1.4–1.66	400.00	0.0005	-	Yes
	D75	2.74	2.43–4.7	207.87	0.0243	-	Yes	3.93	3.36–4.05	1210.00	0.0022	-	Yes
	D105	5.16	4.68–5.23	479.78	0.0003	89.76	Yes	5.46	4.2–5.92	1720.00	0.0019	210.51	Yes
	D135	3.95	3.86–4.54	343.82	0.0000	-	Yes	4.22	3.74–4.38	1306.67	0.0008	-	Yes
	D165	4.36	2.59–5.22	389.89	0.0200	-	Yes	3.94	3.64–4.38	1213.33	0.0001	-	Yes
	D180	3.92	3.59–4.11	340.45	0.0001	-	Yes	3.27	3.03–3.51	990.00	0.0008	-	Yes
p-value serum versus plasma								0.8035					

clinical laboratories prefer using serum as their matrix for analysis [26].

The study revealed the concentration of glucose in the serum and plasma showed significant differences (Table 1). A similar result was reported by Frank et al. [27], who found that the concentrations of serum glucose were significantly lower than those of plasma. Further reported that plasma contains platelets, thus how those cells use glucose can have an impact on how much glucose is measured over time [27]. On the other hand, the serum would be devoid of any cells, making it appropriate for measuring blood glucose, especially when blood glucose is taken after a delay. Additionally, the serum has less protein than plasma, making it cleaner. In some tests, proteins are sometimes regarded as interfering molecules because they interact with the reagent and provide erroneous findings. The blood "lactate threshold," an index of exercise intensity, is thought to be more helpful than heart rate when recommending exercise intensities for most patients, both those with and without the disease [28]. It has been shown that once tubes are centrifuged, lactate concentration increases by 80%–100% in serum or lithium–heparin plasma after 4 hours at 25°C°C [29]. The World Health Organization (WHO) reported that plasma samples are not recommended for the determination of glucose levels [30]. Contrastingly, Butt et al. [31] reported there was no significant difference between mean blood glucose levels in plasma and serum if performed immediately within 30 minutes [31].

The present study found that a significant difference between serum and plasma was found for AST (Table 7). This result was in agreement with Nwosu et al. [32], who reported that for all storage temperatures, the stability of the enzyme in serum was more than that in plasma. An erroneous process for separating serum from components associated with clots [32]. Also, insufficient clotting encourages the lysis of red blood cells, some of which may become caught in gel if present, which may artificially raise serum levels of the analytes most susceptible to hemolysis, such as lactate dehydrogenase (LD) and AST [33,34]. Moreover, differences in the level of several enzymes between serum and plasma, such as AST, alanine aminotransferase, amylase, gamma-glutamyl transferase, creatine kinase, and LD, may be caused by changes in cell permeability or an increase in the fragility of the erythrocyte membrane during clot formation and pre-centrifugation time [22,35]. The World Health Organization (WHO) document states that plasma samples are recommended for AST because the constituents in plasma are better at reflecting the pathologic situation of a patient than those in serum [36]. Contrastingly, the AST did not significantly differ between serum and plasma [37]. The study also found that the mean of AST was a significant variation in storage at D180 in (−20°C) serum samples without clinical impact and D165 and D180 in (4°C) plasma samples with clinical impact. The results were supported by Williams et al. [38], who reported that storage at −20°C for 15 weeks had a mild destructive effect on AST in serum [38]. The plasma AST also significantly decreased enzyme activity at 4°C storage after 42 hours [32]. Contrastingly, the AST showed insignificant changes after 360 days in storage at −20°C of rat serum samples [39].

TB was affected by storage for D180 at either 4°C or −20°C. Longer storage may be associated with a significant loss of stability in serum and plasma, which has a potential clinical impact on serum samples (Table 2). The result was supported by a previous study that reported that the TB stability was decreased when long-term storage. Further reported that there was a significant decrease in TB after storage at −20°C for a month [40]. The results are important to consider measuring the value of TB after a long-time storage will not be accurate. TB stability was evaluated in relation to the pre-centrifugation period and storage temperature of whole blood, serum, and plasma [41]. Bilirubin is photo-sensitive; when exposed to artificial light, it was degraded. The light influenced the bilirubin stability of the plasma and serum [42]. A previous study reported that TB in serum and plasma was stable even over the 56-hour period at room temperature (25 °C) without light exposure [29].

The results indicated clinically significant instability in the serum level of UC at D75 when stored at 4°C (Table 4). A closely similar result was reported by Tambse et al. [43], who observed that the UC starts deteriorating after 32 days stored at 4°C [43]. Another study reported that clinically significant variability was observed in the serum samples stored at −20°C as early as the first month, while statistically significant variability finally occurred in the first, third, and sixth months [44]. This UC observed negligible loss of stability after 45 days of storage compared with a 1-day-old. It showed a decrease in response stability of about 3% compared to the stability of the afresh enzyme electrode observed after storing the electrode for 65 days [45]. The highest stability was shown for the clinical assessment of the UC content during storage at room temperature [46]. In contrast, the

Table 5. Total protein concentration in serum and plasma samples at different preservation times (Day) and temperatures.

		Serum						Plasma					
Analytes	**Stability**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**
Total protein (4°C) (mg/dl)	D0	68.0	67.0–69.9	–	–	–	–	74.9	71.3–79.7	–	–	–	–
	D15	69	66.7–74.2	1.47	0.1061	–	No	71.3	67.9–74.2	−4.81	0.5436	–	No
	D45	89	87.2–94.3	30.88	0.0007	–	No	82.5	78.2–87.0	10.15	0.01	–	No
	D75	73.3	69.0–75.6	7.79	0.1097	–	No	91.0	87.0–95.0	21.50	0.0895	–	No
	D105	65.1	63.7–69.7	−4.26	0.271	31.32	No	69.5	45.8–71.7	−7.21	0.1512	39.66	No
	D135	86.7	79.5–88.2	27.50	0.0205	–	No	81.9	81.2–82.7	9.35	0.3116	–	No
	D165	91	85.3–99.5	33.82	0.0162	–	Yes	78.5	73.4–78.7	4.81	0.916	–	No
	D180	101.7	89.4–104.4	49.56	0.0022	–	Yes	93.5	92.6–95.6	24.83	0.0255	–	No
Total protein (−20°C) (mg/dl)	D0	69.7	67.7–75.6	–	–	–	–	72.7	69.7–75.8	–	–	–	–
	D15	89	81.0–93.3	27.69	0.0202	–	No	76.8	76.3–85.2	5.64	0.1195	–	No
	D45	94.2	78.3–99.2	35.15	0.055	–	No	79.1	76.3–80.4	8.80	0.0105	–	No
	D75	92.9	88.3–94.6	33.29	0.0002	–	No	93.7	91.3–96.5	28.89	0.0009	–	No
	D105	62.3	60.3–62.5	−10.62	0.0447	38.92	No	74.8	67.6–80.3	2.89	0.7283	30.98	No
	D135	85	81.2–87.8	21.95	0.0052	–	No	82.1	80.5–83.9	12.93	0.0016	–	No
	D165	73.8	70.8–84.6	5.88	0.1037	–	No	78.2	74.5–79.6	7.57	0.1452	–	No
	D180	115.6	85–138.6	65.85	0.038	–	Yes	115.5	84.5–126.5	58.87	0.0263	–	Yes
p–value serum versus plasma								0.7807					

Table 6. Cholesterol concentration in serum and plasma samples at different preservation times (Day) and temperatures.

		Serum						Plasma					
Analytes	**Stability**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**
Cholesterol (4°C) (mg/dl)	D0	158.1	79.2–170.2	–	–	–	–	132.4	77.1–158.9	–	–	–	–
	D15	149.2	95.0–175.6	−5.63	0.4757	–	No	143.0	84.3–174.0	8.0	0.0096	–	No
	D45	139.3	84.5–166.2	−11.89	0.7266	–	No	157.6	142.3–162.7	19.0	0.3302	–	No
	D75	160.2	115.2–210.7	1.33	0.0627	–	No	176.8	105.0–181.7	33.5	0.0066	–	No
	D105	188.0	98.0–202.1	18.91	0.0089	–	No	182.4	97.6–197.9	37.8	0.0076	–	No
	D135	143.1	98.9–152.7	−9.49	0.939	130.74	No	146.9	86.9–156.1	11.0	0.3046	127.00	No
	D165	166.9	141.6–171.9	5.57	0.1278	–	No	140.0	101.3–162.5	5.7	0.0479	–	No
	D180	189.7	123.6–196.2	19.99	0.0169	–	No	130.7	121.3–185.8	−6.6	0.0319	–	No
Cholesterol (−20°C) (mg/dl)	D0	112.9	81.1–157	–	–	–	–	137.7	77.1–161.6	–	–	–	–
	D15	107.5	101.1–218.9	−4.78	0.1555	–	No	136.3	103.8–177.3	−1.0	0.1218	–	No
	D45	105.6	91.3–158.1	−6.47	0.9005	–	No	116.5	80.7–153.0	−15.4	0.7912	–	No
	D75	154.9	94.1–174.4	37.20	0.2513	–	No	156.5	91.6–165.4	13.7	0.0271	135.85	No
	D105	177.7	101.1–194.3	57.40	0.0271	140.60	No	165.4	100.5–215.7	20.1	0.0084	–	No
	D135	142.8	84.1–165.7	26.48	0.3867	–	No	124.9	86.1–161.7	−9.3	0.9895	–	No
	D165	145.2	92.5–161.6	28.61	0.042	–	No	131.8	88.9–160.4	−4.3	0.6804	–	No
	D180	173.6	111.8–176.6	53.76	0.0795	–	No	138.1	94.0–143.8	0.3	0.8937	–	No
p–value serum versus plasma								0.3195					

Table 7. AST concentration in serum and plasma samples at different preservation times (Day) and temperatures.

		Serum						Plasma					
Analytes	**Stability**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**	**Median**	**IQR**	**Mean % difference**	***p* value**	**RCV**	**Potential clinical impact**
AST (4°C) (mg/dl)	D0	44.6	43.2–58.8	–	–	–	–	32.6	26.6–50.4	–	–	–	–
	D15	48.9	42.6–64.5	9.64	0.0634	–	No	64.7	64.3–91.7	98.47	0.1108	–	No
	D45	52	50.0–57.9	16.59	0.474	–	No	78.5	55.8–95.8	140.80	0.0345	–	No
	D75	41.9	28.1–59.4	−6.05	0.9968	–	No	48.3	40.6–54.1	48.16	0.3944	–	No
	D105	41	39.9–41.9	−8.07	0.8424	145.46	No	56.5	55.7–67.6	73.31	0.2562	168.08	No
	D135	42.3	38.7–43.2	−5.16	0.7123	–	No	74	53.5–88.6	126.99	0.0315	–	No
	D165	27.6	22.5–27.6	−38.12	0.0579	–	No	85.2	83.7–87.8	161.35	0.0235	–	**Yes**
	D180	43.6	40.1–61.7	−2.24	0.3903	–	No	97	70–105.4	197.55	0.0309	–	**Yes**
AST (−20°C) (mg/dl)	D0	41.8	40.2–64.3	–	–	–	–	53.7	47.5– 55.7	–	–	–	–
	D15	48.6	44.8–70.8	16.27	0.5465	–	No	97.3	88.2–122.4	81.19	0.0224	–	No
	D45	42.5	35.4–45.2	1.67	0.3541	–	No	45.3	36.1–55.5	−15.64	0.6073	–	No
	D75	41.3	34.8–61.9	−1.20	0.771	–	No	52.1	41.9– 59.8	−2.98	0.9264	–	No
	D105	32	29.8–37.3	−23.44	0.273	108.59	No	41.5	38.2–47.0	−22.72	0.2693	90.07	No
	D135	37.3	33.7–45.1	−10.77	0.219	–	No	48.3	37.7–65.1	−10.06	0.8933	–	No
	D165	40.5	36.6–41.7	−3.11	0.0747	–	No	31.9	26.5–42.1	−40.60	0.0341	–	No
	D180	33.8	21.6–37.3	−19.14	0.0456	–	No	49.3	43.9–63.6	−8.19	0.8852	–	No
p–value serum versus plasma								0.0024					

storage sample at −20°C is a better way to preserve UC [4]. Another study reported that higher UC concentrations could be used to make it easier to spike serum samples, which can be kept at temperatures of approximately −18°C [47].

The study revealed clinically significant instability in TP serum levels at D165, D180, and D180 when stored at 4°C and −20°C, respectively, as well as in plasma levels at D180 when stored at −20°C (Table 5). The result was supported by a previous study, which reported that time and temperature have a statistically significant impact on the TP and their fractions [48]. A similar result was reported by a previous study, which found that the median concentration of TP differed significantly between 0 and 6 months at −20°C. During sample storage at 4°C, neither clinically nor statistically significant variability occurred within 120 hours [49]. In the third month of storage, clinically and statistically significant variations in human serum kept at −20°C rendered it useless for measuring TP content [44]. Dissimilar results were reported by a previous research which found that statistically significant variation in TP in both room temperature and refrigerator samples after 14 days [50]. Plasma protein stability was higher than serum protein because the concentration in plasma was slightly higher than in serum. Except for proteins used in clot formation, such as fibrinogen and clotting factors, total serum protein content reflects all the various proteins present in plasma [51]. Contrastingly, the TP remains stable for 90 days in storage at −20°C of rat serum [39].

The present study found that the concentrations of glucose, creatinine, and Chol were clinically insignificant variations in different storage periods at 4°C and −20°C (Tables 1, 3, and 6). These results are supported by several studies [4,5,52]. According to Thoresen et al. [53], those analytes showed statistically significant ($p < 0.05$) changes during the storage period related to storage time and storage temperature of canine serum and plasma without clinical importance [53]. Dissimilar results were reported by Quartey et al. [50], who found that clinically significant changes were seen in creatinine concentrations in human plasma after 7 days [50].

It is necessary to interpret the findings of the analysis of the samples under various storage conditions and timeframes in accordance with the tabulated values. The findings of this study may be very beneficial for identifying the appropriate clinical report interpretation and the time length for sample storage in laboratories.

Conclusion

Serum and plasma are not interchangeable specimens for all biochemical analytes. Serum samples were comparatively better than plasma samples for most of the biochemical analytes, especially glucose and AST. TB clinically significant loss of stability for longer storage time in serum samples but has no potential clinical impact in plasma samples. UC has deleterious effects on any storage temperature and time. It was not suitable for storage in laboratories. TP was more stable in plasma samples than in serum. The results showed that storage samples at −20°C are a better way to preserve TB and TP. Therefore, the samples had to be kept in separate aliquots for re-analysis as the stability of the analytes decreased if thrown and stored again.

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