Veterinary Medicine: Research and Reports

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Stability of Some Biochemical Parameters in Sheep and Goat Serum Stored at −20°C

Yoseph Cherinet Megerssa

Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture Addis Ababa University, Bishoftu, Ethiopia

Correspondence: Yoseph Cherinet Megerssa, Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture, Addis Ababa University, BishOftu, Ethiopia, Tel +251911804383, Email yoseph.cherinet@aau.edu.et

Background: In Veterinary Medicine biochemical investigation of serum is widely used to aid diagnosis and treatment. However, delays usually happen between sampling and analysis. As a result, the serum is stored in refrigerators. In this regard, information on the effects of temperature and storage duration on the stability of the analyte is incomplete in general and its effect in sheep and goat serum is not described. Therefore, the objective of this study was to examine the stability of selected biochemical analytes from sheep and goat serum following storage at −20°C for 2 months.

Methods: Serum from 20 apparently healthy male 2–2.5 year-old sheep and goats was obtained and aliquots of serum from each sample were kept in three tubes. The first tube is for baseline (T0), which is done within an hour, while the other two (T1 and T2) are stored at −20°C for 1 and 2 months, respectively. Total protein, albumin, urea, total cholesterol, and triglycerides were assayed.

Results: The results revealed that storage temperature and duration for up to 2 months had no significant effect on any analytes except for urea in goats. The changes in terms of total observed error (TE_o) for total protein; albumin and urea were greater than the acceptable values in both animals.

Conclusion: Thus, further studies are required to assure alteration of analyte at various storage temperatures and duration. In addition, implementation of quality systems to achieve quality targets for analytes with greater TE_o as compared to the established TE_a is needed.

Keywords: biochemical, goat, serum, sheep, stability

Introduction

Biochemical investigation is widely used to aid the diagnosis and treatment of animals; however, a delay usually occurs between sampling and analysis.[1] Samples are usually stored in refrigerators due to various reasons including the use or re-use of samples is needed due to delay in the analysis procedure; to confirm or to check a previously obtained value, to add new quantifications of missing analytes and when samples are transferred to distant places.[2,3] Hence, stability of analytes following storage should be noted as it is an important pre-analytical factor that determines the accuracy of results.[4]

It is difficult to apply recommendations from the World Health Organization (WHO) and Clinical Laboratory Standard Institute (CLSI) in routine practice as the analyte stability described is often not compatible with the time taken to transport blood samples from the place of collection to the laboratory.[5,6] There is variation among analytes instability; therefore, different storage conditions may apply depending on their nature of storage.[7] Stability studies of analytes after storage compared with the fresh sample values, with an estimation of recovery rates, are important to determine the effects of storage. Recovery rates may increase or decrease after storage.[8] Thus, storage temperature and duration are the essential parameters that must be considered in order to maintain the composition and integrity of representative samples during the pre-analytical phase.[9,10]

Published literature pertaining to chemical analyte stability has addressed many issues related to samples but largely on human serum.[2,10–13] There is limited information available regarding the stability of analytes in animals in particular to sheep and goat serum.[14–16] Therefore, the present study examined the effect of storage on the stability of five selected biochemical analytes in serum following storage at −20°C for 2 months.

Received: 26 September 2022
Accepted: 16 November 2022
Published: 21 November 2022

Materials and Methods

Study Animals and Sampling Technique

Twenty adult male sheep and goats of 2–2.5 years of age were recruited from the farm of the College of veterinary medicine and Agriculture. The study animals were apparently healthy and blood samples were collected. Animals with a history of medication were excluded due to the possible impact of drugs on biochemical analysis. The use of animals for study and sampling was approved by the institutional animal research ethics committee at the Addis Ababa University College of veterinary medicine and agriculture (Certificate No VM/ERC/04/14/022).

Collection and Processing of Blood Samples

Serum was obtained from sheep and goats following collection of 5 mL blood samples by a veterinarian from the jugular vein. Blood was left to clot at room temperature for 30 minutes. Clotted blood was centrifuged at 1,200×g for 10 minutes and aliquots of serum were divided into three labeled Eppendorf Safe-Lock tubes as T0, T1, and T2. The two Eppendorf safe-locks (T1 and T2) were stored in a refrigerator at −20°C while the T0 was used for day one (baseline), which stipulated analysis within an hour. The samples stored at −20°C (T1, and T2) were analyzed after bringing them to room temperature for approximately 1 hour until completely thawed, and then mixed properly with automatic pipettes before analysis.

Laboratory Methods

Biochemical parameters were analyzed by semi-automated chemistry analyzer EMP-168 Chengdu Empsun Medical Technology Co., Ltd (AMP Diagnostics, USA). The methods utilized for biochemical analysis include total protein by end point biuret, albumin by end point bromocresol green (BCG), urea by kinetic urease/GLDH (Glutamate dehydrogenase), total cholesterol by end point cholesterol peroxidase4-aminophenazone (CHOD-PAP), and triglycerides by end point enzymatic determination by glycerol phosphate oxidase and peroxidase (GPO/PAP).[17] Quality control (QC) was made each day before (Jouri labs diagnostic reagent and stains Addis Ababa Ethiopia) running the samples.

Data Analysis

The stability of analytes was assessed using statistical parameters and comparison to established allowable total error for biochemical analytes. IBM SPSS V.20 was used for statistical analysis. The normality of the data was tested using the Kolmogorov–Smirnov, and data obtained at the T0, T1, and T2 were analyzed using repeated measures ANOVA to compare the overall difference between the means at storage times T0, T1, and T2. The results are expressed as means±SD. A *P*-value of less than 0.05 was considered as statistically significant. Post-hoc analysis was assessed with a Bonferroni adjustment to ensuing a significance level at $P<0.017$ (0.05/3=0.017) was set for each analyte regarding time interval (T0, T1, and T2).

For total allowable error (T_a); bias (%) for T1 and T2 was calculated from percentage change from baseline (T0) values and the %CV for each analyte was calculated by finding the SD of results T0 and T1, T0 and T2, dividing that by the duplicate mean and multiplying by 100. The average of the individual CVs reported as the intra CV. TE_o (%) was calculated as 2CV (%) + bias (%). The TE_a (%) used in the current study were adopted from the American Society of Veterinary Clinical Pathology (ASVCP) guidelines: allowable total error guidelines for biochemistry. If TE_o (%) is less than TE_a (%), the quality requirement passes and no further action is needed.[18]

Results

The study demonstrated the effect of storage on the stability of some biochemical analytes in serum harvested from sheep and goats. Five analytes, namely total protein, albumin, urea, total cholesterol, and triglycerides, were studied. Baseline values (T0) and later, following storage at −20°C for 1 month (T1) and 2 months (T2), the analytes were determined. Repeated measures ANOVA were utilized to describe the overall difference between the means at T0, T1, and T2 time points. To this effect Mauchly's test was done and if $P>0.05$ it meet the assumption of sphericity and its value taken. While those violated the assumption of sphericity, *P*-value from Greenhouse-Geisser correction was taken. As shown below all parameters except urea in goats and total cholesterol in both animas were not statistically significant (Table 1).

Table 1 Repeated Measures of ANOVA Values for Analytes in Sheep and Goat

Analytes	Animal	N	Values of Analytes Mean±SD			F-Value	*P*-value
			T0	T1	T2		
Total Protein (g/dL)	Sheep	20	6.95±0.69	6.79±0.67	6.63±0.82	8.548	0.310
	Goat	20	6.49±0.87	6.31±0.69	6.21±0.48	2.542	0.119
Albumin (g/dL)	Sheep	20	3.30±0.20	3.37±0.24	3.52±0.21	10.584	0.358
	Goat	20	3.47±0.39	3.32±0.45	3.29±0.47	1.592	0.223
Urea (mg/dL)	Sheep	20	33.24±6.60	31.96±5.95	32.58±6.44	3.213	0.145
	Goat	20	40.67±4.92	38.10±6.53	37.28±5.74	5.377	0.016
Total Cholesterol (mg/dL)	Sheep	20	34.71±9.41	35.32±10.41	37.79±10.25	3.855	0.030
	Goat	20	50.26±10.26	48.26±9.36	49.03±9.75	3.530	0.039
Triglycerides (mg/dL)	Sheep	20	35.04±9.94	36.43±9.66	36.25±8.27	1.394	0.068
	Goat	20	37.18±18.52	35.90±17.88	37.72±18.10	1.957	0.155

For parameters with statistical significance, a post-hoc pairwise comparison using the Bonferroni correction showed decreased stability of total cholesterol with no statistical significance between T0 and T1 (50.27 vs 48.26, respectively), P=0.051 and, while between T0 and T2 (50.27 vs 49.04, respectively), P =0.384 were observed in goat. In sheep there was decreased stability with no statistical significance between T0 and T1 (34.71 vs 35.32, respectively), P =1.000, and between T0 and T2 (34.71 vs 37.79 respectively), P=0.040 were noted. For urea; statistical significance between T0 and T1 (40.67 vs 38.10, respectively), P=0.016 and between T0 and T2 (40.67 vs 37.28, respectively), P=0.010 was noted in goat samples.

The study also demonstrated the changes of analyte values after storage in terms of TE_a (%) adopted from the American Society of Veterinary Clinical Pathology (ASVCP) guideline: allowable errors for biochemical analytes. The observed changes or total observed errors (TE_o) were estimated by adding the random error (% CV) from intra-assay of T0–T1 and T0–T2, while systematic error (bias%) from percentage change of T1 and T2 from baseline values (T0). A comparison of the current findings with the published values of allowable error[18] indicated that total cholesterol and triglycerides of both species were within an acceptable range. Albumin was also within an acceptable range only in goat samples. Other parameters were not within an acceptable range (Table 2).

Discussion

In laboratory testing, the pre-analytical phase is the most critical part of the analytical process that has an impact on results.[19] One of the factors in the pre-analytical phase is storage.[20,21] Although the use of freshly collected serum is recommended, delay in the testing process, reuse of samples for missing results, and when samples are transferred from distant places is inevitable.[22] Therefore, this study was undertaken to examine the stability of some biochemical analytes, namely total protein, albumin urea, total cholesterol, and triglycerides following storage of sheep and goat serum at −20° C for different periods; T0 (baseline), T1 (1 month), and T2 (2 Months). The study demonstrates that all except urea were stable for up to 2 months when stored at −20°C, which is in line with the recommendation of long-term storage of analytes storage −20°C.[23] To the author's knowledge, the study was the first report in the veterinary laboratory setting in Ethiopia that studied the stability of biochemical analytes in sheep and goat serum following storage at −20°C. Published literature pertaining to analyte stability has addressed limited information regarding the stability of analytes in animals, in particular to sheep and goat serum. The term "Instability" of biochemical analytes in plasma during storage does not always suggest a reduction in concentrations of the analytes, in some cases rather unexplained increases may happen and mechanisms for such changes need to be explored further.[24]

Table 2 Changes of Analyte Values After Storage in Terms of TE_a (%) in Sheep and Goat

Analytes	Animal	N	Percentage Change From Baseline						TE_a (%)
			T1			T2			
			CV (%)	Bias (%)	TE_o (%)	CV (%)	Bias (%)	TE_o (%)	
Total Protein(g/dL)	Sheep	20	2.14	2.30	6.58	4.18	4.60	12.96	10%
	Goat	20	2.49	2.77	7.75	4.75	4.31	13.81	
Albumin (g/dL)	Sheep	20	3.42	2.12	8.96	5.00	6.66	16.66	15%
	Goat	20	8.77	4.32	21.86	9.63	5.18	24.44	
Urea (mg/dL)	Sheep	20	4.27	3.85	12.39	5.02	1.98	22.06	12%
	Goat	20	7.00	6.32	20.32	8.02	8.33	24.37	
Total Cholesterol (mg/dL)	Sheep	20	1.66	8.08	11.40	1.88	6.13	9.89	20%
	Goat	20	4.50	3.98	12.98	4.47	2.45	11.39	
Triglycerides (mg/dL)	Sheep	20	6.89	3.97	17.75	8.08	3.45	19.61	25%
	Sheep	20	5.26	3.44	13.96	7.26	1.45	15.97	

Abbreviations: TE_o, Observed total error; TE_a, Allowable total error.

Different reports have mentioned the analyte stabilities in similar or different ways. Contrary to the current study, Cuhadar et al[25] noted significant variations of total protein in sera stored until 3 months. On the other hand, Kovačević et al[26] described the stability of total protein up to 6 months when stored at −20°C. Similar to the current study, significant variations of urea in sera stored until 3 months were observed by Cuhadar et al.[25] On the other hand, Kovačević et al[26] described the stability of urea till 5 and 6 months when stored at −20°C. The stability of total cholesterol and triglycerides was mentioned by Paltiel et al [27] and Comstock et al.[28] Kovačević et al[26] also noted that total cholesterol and triglycerides were stable at −20°C for 5 months and 4 months, respectively, regardless of pre-analytical factors. Similar to our findings, the stability of albumin was also noted by Maxwell et al[29] weekly over a 5-week period. Kovačević et al[26] also described the stability of albumin for 6 months when stored at −20°C. However; significant variability of albumin after storage at −20°C from month to 6 months was noted by Pawlik-Sobecka et al.[30]

In clinical medicine, veterinarians are interested to have valid data on laboratory results. Thus, it is good to have knowledge about the accuracy and impression of the results, which combined as TE_a.[31] The current revealed that most were not within an acceptable range and as no adequate and published reports in veterinary medicine in these regard, we are unable to discuss the findings of the current study. The allowable total error suggested in ASVCP guidelines is general and, thus, it might not reflect the acceptable biological and analytical range suitable for sheep and goats; hence, determination of species-based allowable total error is needed.

Conclusion

Storage of serum is unavoidable when needing to confirm previous results or perform additional analysis. There are differences among the studies in analyte stability and our findings which might be due to differences in sample type, serum separation, storage temperatures, storage duration, and test methodologies. This study is the first report on the stability of biochemical analytes in sheep and goat serum in a veterinary laboratory setting in Ethiopia. All analytes except urea in goats under the study were stable for 2 months when stored at −20°C. However, further studies are necessary to assure any alterations in the results of biochemical analytes when stored at −20°C and at various storage temperatures and durations. In addition, implementation of quality systems and risk management guidelines to achieve quality targets for those analytes (total protein, albumin and urea) with greater TE_o as compared to the established TE_a are needed.

Limitations of the Study

- The study was unable to conduct stability studies in a wide range of temperatures and durations. Only some biochemical parameters were studied due to a lack of test kits.
- The study used apparently healthy animals aged 2–2.5 years and it would be good to also assess the stability from sick animals of different pathological conditions across different ages and a broader analytical range.

Abbreviations

AAU, Addis Ababa University; ANOVA, Analysis of Variance; ASVCP, American Society of Veterinary Clinical Pathology; CLSI, Clinical Laboratory Standard Institute; SD, Standard Deviation; SPANA, Society for Protection of Animals Abroad; TE_a, Total Allowable Error; TE_o, Total Observed Error; WHO, World Health Organization.

Data Sharing Statement

The data used to support this study are available from the corresponding author on request.

Ethics Approval and Consent to Participate

The study protocol obtained research ethical clearance approved by the institutional animal research ethics committee at the Addis Ababa university college of veterinary medicine and agriculture used in this study (Certificate reference no VM/ERC/04/14/022). Verbal informed consent was also obtained from the owners of animals to collect samples as suggested by the institutional animal research ethics review committee and animals were treated with best practice of veterinary care.

Consent to Publish

Not applicable. The study participants were sheep and goats; however, verbal informed consent was obtained from the owners of the study animals to publish the finding of the study.

Acknowledgment

The author would like to thank Fikru Regassa (PhD), Melaku Yayehirad (MSc) Dereje Bekele (BSc), Tolosa Gutema (BSc), and Meskerem Mullisa (BSc) staffs of the Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture Addis Ababa University for their cooperation during research.

Author Contributions

The author made significant contribution to the work reported here whether that is in study conception, study design, execution, acquisition of data, and data analysis, drafting or revising the article, has agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding

This research was supported by the Addis Ababa University research and technology transfer office through an adaptive problem-solving research fund (Reference no: RD/PY-147/2021, Date 20-September-2021). The author has no financial conflict of interest to declare.

Disclosure

The author declares no competing interests in relation to this work.

References

1. Hulme-Moir KL, Clark P, Spencer PB. Effects of temperature and duration of sample storage on the haematological characteristics of western grey kangaroos (Macropus fuliginosus). *Aust Vet J*. 2006;84:143–147. doi:10.1111/j.1751-0813.2006.tb13400.x
2. Gislefoss RE, Grimsrud TK, Mørkrid L. Stability of selected serum hormones and lipids after long-term storage in the Janus Serum Bank. *Clin Chem*. 2015;48:364–369.

3. Mijovic V, Contreras M, Barbara J. Serum alanine aminotransferase (ALT) and γ-glutamyltransferase (γ-GT) activities in north London blood donors. *J Clin Pathol*. 1987;40:1340–1344. doi:10.1136/jcp.40.11.1340
4. Kachhawa K, Kachhawa P, Varma M, Behera R, Agrawal D, Kumar S. Study of the stability of various biochemical analytes in samples stored at different predefined storage conditions at an accredited laboratory of India. *J Lab Physicians*. 2017;9(1):11–15. doi:10.4103/0974-2727.187928
5. Quality of diagnostic samples. Recommendations of the working group on preanalytical quality of the German society for clinical chemistry and laboratory medicine; 2009:85.
6. Clinical and Laboratory Standards Institute (CLSI). Procedures for the handling and processing of blood specimens for common laboratory tests; approved guideline; 2010.
7. Rifai N, Horvath AR, Wittwer CT. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 6th ed. Elsevier; 2018:e1–196.e38.
8. Brinc D, Chan MK, Venner AA, et al. Long-term stability of biochemical markers in pediatric serum specimens stored at −800 C: a caliper Sub study. 2012. *Clin Biochem*. 2012;45:816–826. doi:10.1016/j.clinbiochem.2012.03.029
9. Stokol T, Nydam V. Effect of anticoagulant and storage condition on bovine non-esterified fatty acid and bhydro- xybutyrate concentration in blood. *J Dairy Sci*. 2005;88:3139–3144. doi:10.3168/jds.S0022-0302(05)72996-9
10. Gislefoss RE, Lauritzen M, Langseth H, et al. Effect of multiple freeze-thaw cycles on selected biochemical serum components. *Clin Chem Lab Med*. 2017;55:967–973. doi:10.1515/cclm-2016-0892
11. Ikeda K, Ichihara K, Hashiguchi T, et al. Evaluation of the short-term stability of specimens for clinical laboratory testing. *Biopreserv Biobank*. 2015;13:135–143. doi:10.1089/bio.2014.0072
12. Zander J, Bruegel M, Kleinhempel A, et al. Effect of biobanking conditions on short-term stability of biomarkers in human serum and plasma. *Clin Chem Lab Med*. 2014;52:629–639. doi:10.1515/cclm-2013-0705
13. Jansen E, Beekhof P, Viezeliene D, Muzakova V, Skalicky J. Longterm stability of cancer biomarkers in human serum: biomarkers of oxidative stress and redox status, homocysteine, CRP and the enzymes ALT and GGT. *Biomarkers*. 2015;9:425–432. doi:10.2217/bmm.15.14
14. Hawkins MG, Kass PH, Zinkl JG, Tell LA. Comparison of biochemical values in serum and plasma, fresh and frozen plasma, and hemolyzed samples from Orange-winged Amazon parrots (Amazona amazonica). *Vet Clin Pathol*. 2006;35:219–225. doi:10.1111/j.1939-165X.2006.tb00118.x
15. Reynolds B, Taillade B, Medaille C, Palenche F, Trumel C, Lefebvre HP. Effect of repeated freeze-thaw cycles on routine plasma biochemical constituents in canine plasma. *Vet Clin Pathol*. 2006;35:339–340. doi:10.1111/j.1939-165X.2006.tb00144.x
16. Thoresen SI, Havre GN, Morberg H, Mowinckel P. Effects of storage time on chemistry results from canine whole blood, heparinized whole blood, serum, and heparinized plasma. *Vet Clin Pathol*. 1992;21:88–94. doi:10.1111/j.1939-165X.1992.tb00591.x
17. Burtis CA, Edward R. *Tietz: Fundamentals of Clinical Chemistry*. Philadelphia: Elsevier Health Sciences; 2001.
18. Harr KE, Flatland B, Nabity M, Freeman KP. ASVCP guidelines: allowable total error guidelines for biochemistry. *Vet Clin Pathol*. 2013;42 (4):424–436. doi:10.1111/vcp.12101
19. Kaplan LA. Determination and application of desirable analytical performance goals: the ISO/TC 212 approach. *Scandivian J Clin Lab Investi*. 1999;59:479–482. doi:10.1080/00365519950185193
20. Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. *Clin Chem Lab Med*. 2006;44:358–365. doi:10.1515/CCLM.2006.073
21. Lippi G, Chance JJ, Church S, Dazzi P, Fontana R, Giavarina D. Preanalytical quality improvement: from dream to reality. *Clin Chem Lab Med*. 2011;49:1113–1126. doi:10.1515/CCLM.2011.600
22. Lippi G, Plebani M, Simundic AM. Quality in laboratory diagnostics: from theory to practice. *Biochem Med*. 2010;20:126–130. doi:10.11613/BM.2010.014
23. Cray C, Rodriguez M, Zaias J, Altman NH. Effects of storage temperature and time on clinical biochemical parameters from rat serum. *J Am Assoc Lab Anim Sci*. 2009;48(2):202–204.
24. Quartey P, Perez Q, James OT. Yawo SR stability of selected biochemical analytes in plasma samples stored under different time and temperature conditions. *J Clin Chem Lab Med*. 2018;1:115.
25. Cuhadar S, Koseoglu M, Atay A, Dirican A. The effect of storage time and freeze-thaw cycles on the stability of serum samples. *Biochem Med*. 2013;23(1):70–77. doi:10.11613/BM.2013.009
26. Cincović KM, Belić B, Đoković R, Majkić M. Blood serum stability limit and maximum storage time of bovine samples. *Acta Scientiae Veterinariae*. 2021;49:1815.
27. Paltiel L, Rønningen KS, Meltzer HM, Baker SV, Hoppin JA. Evaluation of freeze thaw cycles on stored plasma in the Biobank of the Norwegian Mother and child cohort study. *Cell Preserv Technol*. 2008;6:223–230. doi:10.1089/cpt.2008.0012
28. Comstock GW, Burke AE, Norkus EP, Gordon GB, Hoffman SC, Helzlsouer KJ. Effects of repeated freeze-thaw cycles on concentrations of cholesterol, micronutrients, and hormones in human plasma and serum. *Clin Chem*. 2001;47:139–142. doi:10.1093/clinchem/47.1.139
29. Akonde M, Antuamwine BB, Quaye L, Abdul-Mumin Z. Assessing the stability of human albumin under storage in the blood transfusion bank for therapeutic interventions. *Am J Clin Pathol*. 2008;150(1):S141. doi:10.1093/ajcp/aqy105.337
30. Pawlik-Sobecka L, Sołkiewicz K, Kokot I, et al. The influence of serum sample storage conditions on selected laboratory parameters related to oxidative stress: a preliminary study. *Diagnostics*. 2020;10(1):51. doi:10.3390/diagnostics10010051
31. Oosterhuis WP. Gross overestimation of total allowable error based on biological variation. *Clin Chem*. 2011;57:1334–1336. doi:10.1373/clinchem.2011.165308