Effect of Temperature and storage time on Hepatobiliary enzyme activities in Goat serum

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Abstract

The present study was designed and conducted to choose an ideal storage condition for goat sera samples meant for the assay of hepatobiliary enzymes such as, alanine aminotransferases(ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) and gamma glutamyltransferases (GGT) by storing at room temperature, 4 ºC and -20 ºC up to 14 days. Gamma glutamyltransferase was found to be the most stable enzyme in all the three storage conditions through out the study period. Alanine aminotransferase was stable only up to 8 days at 4 ºC whereas marked stability was noticed at -20 ºC and room temperature as long as 14 days. Aspartate aminotransferase was more stable at -20 ºC up to14 days and 11 days at 4 ºC whereas at room temperature only 2 days. Alkaline phosphatase showed great variation upon storage as compared to other hepatobiliary enzymes and it is suggested that its estimation should be performed in fresh serum samples to get a more accurate result. From these results it is therefore advisable to consider stability of each serum hepatobiliary enzymes for different animals separately before preserving sera samples to get more valid and reliable result.

Key words: storage stability, crossbred goats, hepatobiliary enzymes

Introduction

The measurement of serum enzymes is an important tool for disease diagnosis in veterinary and human clinical practice. The routinely used enzymes to evaluate hepatic damage in animals includes ALT, AST, ALP, GGT, Sorbitol dehydrogenase (SDH), Lactate dehydrogenase (LDH), Ornithine carbamoyl transferase (OCT) and 5' Nucleotidase (NTP) (Kaneko et al., 2008). When large numbers of blood samples are collected or when many different analysis are required it is inevitable to store the samples. A general problem facing in clinical laboratories is the loss of enzyme activity during preservation. Refrigeration and freezing preserve most of the enzymes but some deteriorate even when frozen. Stability of an enzymes activity in one species does not mean stability in a second. Many investigations have been undertaken on the stability of enzymes, in vitro, but the results are widely divergent (Kaplan and Pesce, 1989).

In veterinary medicine, to date not much studies have been published on the stability of biochemical markers especially serum hepatobiliary enzymes, which are routinely analysed for clinical diagnostic use. Hence, the present study is taken up to evaluate the effect of storage time and temperature on the measured activities of the hepatobiliary enzymes in the sera samples of goat under various storage condition viz at room temperature, 4 ºC and -20 ºC for a period of two weeks.

Materials and Methods

Eight healthy female crossbred goats of age 3 to 5 years maintained at University Goat and Sheep Farm, College of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy, Thrissur were selected randomly for the study. Blood samples were collected by jugular venipuncture using sterile needles (18 gauge) directly into clean dry sterile glass tubes without anticoagulants. Serum was harvested after 30 to 45 minutes following clot formation and by centrifugation for 10 minutes at 2000 g.

The clear serum was immediately assayed for the following hepatobiliary enzymes ALT, AST, ALP and GGT within an hour of serum separation to serve as basal fresh values (day 0). The remaining serum were dispensed into 18 sample tubes, closed tightly and divided into three groups. One of each group was stored upright at room temperature (approximately 25

Table 1. Activity of ALT and AST in goat sera samples preserved at 25 ºC, 4 ºC and -20 ºC for 14 days

Days of Storage	ALT			AST		
	25 ºC	4 ºC	-20 ºC	25 ºC	4 ºC	-20 ºC
0	16.70±1.80	16.70±1.80	16.70±1.80	74.67±2.60	74.67±2.60	74.67±2.60
1	16.50±2.17 (-1.19)	17.80±2.15 (+6.58)	15.80±1.47 (-5.39)	75.17±2.99 (+0.67)	76.20±3.03 (+2.05)	80.00±3.77 (+7.14)
2	16.70±2.29 (0)	17.80±1.30 (+6.58)	14.70±2.32 (-11.98)	74.33±5.65 (-0.46)	76.00±3.13 (+1.77)	79.80±3.23 (+6.87)
5	15.30±2.04 (-8.38)	20.70±1.43 (+23.95)	16.50±2.14 (-1.19)	53.33±6.17* (-28.58)	75.80±2.29 (+1.51)	74.50±4.49 (-0.23)
8	13.20±0.83 (-20.95)	17.00±1.61 (+1.79)	17.00±1.03 (+1.79)	48.67±3.79* (-34.82)	70.80±3.59 (-5.18)	77.70±3.36 (+4.06)
11	12.70±1.45 (-23.95)	20.80±1.54* (+24.55)	19.80±1.30 (18.56)	43.17±8.78* (-42.19)	82.30±4.15 (+10.22)	73.50±2.93 (-1.57)
14	14.30±0.96 (-14.37)	21.00±2.13* (+25.75)	19.70±3.11 (+17.96)	37.50±6.88* (-49.78)	89.70±5.34* (+20.13)	71.60±4.01 (-4.11)

ºC), 4 ºC and -20 ºC. The stored serum aliquots from all temperature and time points were analysed together in one batch for hepatobiliary enzymes on 1, 2, 5, 8, 11 and 14 days post collection. Prior to analysis, at each designated time, the aliquots of the frozen samples were left to stand at room temperature to thaw and inverted several times to mix. The enzyme assay was performed using Ecoline-Merck diagnostic kits (Merck Specialities Pvt. Ltd, Mumbai) on an automated blood analyzer (Microlab 200).

To test the significant differences in enzyme activity between storage temperatures and to assess the significant trends over time at each temperature, the data was analysed statistically using paired t test (Snedecor and Cochran, 1994). The stability of an enzyme activity under each temperature condition and time was determined by calculating the percentage change in concentrations from the mean fresh value (day 0) at each time-point for each animal.

Results and Discussion

The time and temperature had significant effects on hepatobiliary enzyme activity in serum samples during their storage (Table 1 and 2). Gamma glutamyl transferase was found to be the most stable enzyme in goat serum being stable for 14 days under all the storage conditions studied. A negligible increase in GGT activity was observed at 25 ºC and 4 ºC, but at -20 ºC, about 4 to 14 % decrease noticed from 2nd to 14th day of storage, but were not significant. The ALT was more stable in serum stored at room temperature, retaining about 86 % of the initial activity on 14th day of preservation. At 4 ºC the ALT activity increased with about 25 % excess of initial activity at the end of experimental period. Significantly higher variation in activity was noticed after 8 days of storage (P= 0.05). The ALT values for the samples stored at -20 ºC decreased during the initial period of investigation, followed by an increase from 8th day to 14th day (+17 to +18 %), but changes were not statistically significant.

The AST activities were stable for 2 weeks in serum samples stored at -20 ºC without any significant variation. At room temperature, AST activities remained unaltered for 2 days and then gradually decreased, remaining only 50 % of the initial activity on 14th day. Loss of activity was significant from 5th day onwards (P= 0.05). Much variation in its activity were not observed at 4 ºC up to 8th day but thereafter the activity gradually increased with a significant higher level on 14th day.

Alkaline phosphatase was considered to be unstable in serum preserved at room temperature (25 ºC) because of significant decrease in activity within 24 h of venipuncture and also due to great variation in the enzyme activity. Negligible decrease in ALP activity was found initially at 4 ºC, followed by an increased activity which was significant after 8 days. Much variation in ALT activity were detected in serum kept at -20 ºC, where the activity decreased during the first 2 days, then increased and finally the activity decreased significantly after 5th day of preservation.

Sera preservation at -20 ºC is also recommended for AST assay. Changes in the temperature of storage alter the rate of reaction and rate of denaturation of enzymes. Intracellularly, enzymes are protected from degradation when bound to their substrates and cofactors. In serum, enzymes, substrates and cofactors are dispersed and binding is uncommon, leaving the enzymes more susceptible to degradation (Kaplan and Pesce, 1989). In the present investigation, the most unstable enzyme was found to be ALP. Its activity varies significantly within one day at room temperature and after 5th or 8th day at -20 ºC and 4 ºC, respectively. A tendency to increase in ALP activity

Table 2. Activity of ALP and GGT in goat sera samples preserved at 25 ºC, 4 ºC and -20 ºC for 14 days

Days of Storage	ALT			AST		
	25 ºC	4 ºC	-20 ºC	25 ºC	4 ºC	-20 ºC
0	166.70±13.92	166.70±13.92	166.70±13.92	36.40±3.04	36.40±3.04	36.40±3.04
1	157.20±13.36* (-5.69)	151.80±11.54 (-8.94)	140.60±8.96 (-15.66)	38.60±3.27 (+6.04)	35.60±2.63 (-2.19)	38.20±3.39 (+4.95)
2	167.60±14.02 (+0.54)	160.10±22.34 (-3.96)	147.40±18.22 (-11.58)	36.40±3.19 (0)	37.20±2.39 (+2.19)	34.80±3.04 (-4.39)
5	194.40±17.04 (+16.63)	209.00±21.90 (+25.37)	177.40±33.49 (+6.42)	39.80±3.09 (+9.34)	38.80±3.52 (+6.59)	34.40±3.69 (-5.49)
8	199.60±21.96 (+19.75)	213.10±21.70 (+27.83)	122.00±14.52* (-26.81)	37.20±2.46 (+2.19)	37.00±3.18 (+1.64)	31.40±2.93 (-13.74)
11	210.10±24.56 (+26.03)	245.00±32.63* (+49.97)	123.10±29.05* (-26.16)	38.20±2.52 (+4.95)	37.00±2.12 (+1.64)	31.80±3.37 (-12.64)
14	213.60±26.61 (+28.13)	209.60±26.38 (+25.73)	164.30±18.42 (-1.44)	38.80±1.96 (+6.59)	36.60±2.71 (+0.55)	31.20±2.53 (14.29)

was noticed in serum samples stored at room temperature and 4 ºC. ALP in human serum also demonstrates a linear increase in activity dependent on temperature and time (Kaplan and Pesce, 1989). The increased activity may be due to the occurrence of several isoenzymes for ALP which differs in their stability to temperature.

Alanine aminotransferase exhibited greater stability at room temperature and -20 ºC. This is in contrary to the findings of Davy et al. (1984), they reported ALT to be highly unstable in marmoset plasma at -20 ºC. In human serum, ALT activity was also reported to be highly unstable at freezing (Kaplan and Pesce, 1989). This reveals species specific differences in the stability characteristics of ALT. At 4 ºC, the enzyme was stable up to 8 days of preservation and a marked increase in activity was noticed thereafter. A similar result was observed in case of camel serum stored at 4 to 5 ºC (Saeed et al., 1995). The results suggest -20 ºC as the ideal storage condition for the preservation of goat sera samples for ALT assay.

The decline in AST activity noticed for samples at room temperature may be due to increased degradation of enzyme active site with increased temperature. These observations were consistent with similar studies conducted by Lazaroni et al. (1958); Heins et al. (1995) and Saeed et al. (1995) on human, marmoset and camel serum, respectively. Among the four hepatobiliary enzymes studied, GGT was found to be more stable up to the end of the study period under the three storage conditions. The results were in accordance with the study of Donnley et al. (1995) on human serum and they stated that GGT to be highly stable at 4 ºC for 2 weeks and up to 4 months at -20 ºC.

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