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ORIGINAL RESEARCH

Is it possible to use expired tubes for routine biochemical analysis in dogs?

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Key Words
Canine, clinical chemistry, expiration date, heparin, plasma

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Background: Expired collection tubes may be used inadvertently and resampling is not always possible. To date, studies have not been conducted in veterinary medicine to determine whether or not biochemical measurements obtained from specimens collected into expired tubes are accurate enough for clinical decision-making.
Objectives: The aims of this preliminary study were to assess the impact of measuring routine plasma biochemical analytes in canine specimens collected in expired tubes and to investigate the relationship between post-expiration time and the magnitude of errors.
Methods: Blood specimens were collected from 61 dogs and aliquoted equally into tubes containing lithium heparin and gel. One tube was within the expiration date, and the other tube was up to 11 months post-expiration. Plasma was separated within 1 hour of specimen collection, and concentrations of urea, creatinine, total protein, albumin, total bilirubin, cholesterol, triglycerides, magnesium, calcium, phosphates, sodium, potassium, chloride, total CO_2, and fructosamine and activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (ALP), γ-glutamyltransferase, lactate dehydrogenase, creatine kinase, amylase, and lipase were analyzed immediately and results compared.
Results: For most analytes there was no significant difference between results from specimens collected in non-expired and expired tubes. For ALP and lipase activities and fructosamine and total CO_2 concentrations, significant differences were found, and results obtained for fructosamine and total CO_2 from specimens in expired tubes may have led to erroneous interpretations. The effect of time since expiration was constant over time.
Conclusions: When specimens are processed within 1 hour of collection, results of routine biochemical measurements of blood collected in lithium heparin tubes remain clinically valid for up to 11 months after expiration of tubes for the majority of analytes, except for ALP, lipase, fructosamine, and total CO_2.

Introduction

In human medicine, the most common causes of variation in laboratory medicine occur during the preanalytical phase,[1] and it is likely that this is also true for veterinary medicine. Errors may occur at any time from specimen collection to submission to the laboratory to processing of the specimen by the laboratory.[2] In veterinary medicine, the preanalytical phase is controlled primarily by the clinician or veterinary technician, who should ensure that all preanalytical procedures are standardized and conducted in accordance with laboratory requirements.

Commercial collection tubes, especially tubes containing anticoagulants such as heparin, EDTA, or citrate, have expiration dates to ensure they are suitable for use and to maintain desired quality. Expiration dates, an important quality control factor, may not be checked prior to specimen collection, and it is not unusual for a laboratory to receive specimens in expired

tubes. It is not always possible to collect another specimen, and, thus, it is important to know the impact of using expired tubes on laboratory results. In a study of ethanol measurement in human medicine, use of tubes that had expired up to 74 months prior to specimen collection had no impact on results.[3] Although many studies in veterinary medicine have reported the influence of storage time, temperature, repeated freeze-thaw cycles, and different anticoagulants on plasma analytes,[4–8] to our knowledge a study of the effect of using expired tubes has not been reported.

In this preliminary study, we chose to limit our investigation to the use of heparin-lithium tubes with gel separator for biochemical analysis of plasma. Plasma is often preferred to serum for routine biochemical analysis; it can be obtained more quickly, as there is no need to wait for clot formation and centrifugation velocity can be increased, and a larger volume is obtainable, which is particularly important for specimens from small animals.[9] In addition, anticoagulation prevents formation of small clots that may interfere with analysis.[9] Finally, the risk of hemolysis and lysis of platelets is reduced.[9] The purpose of this preliminary study was to assess the impact of measuring routine plasma biochemical analytes in canine specimens collected in expired tubes and to investigate the relationship between post-expiration time and the magnitude of errors.

Materials and Methods

The study was conducted from November 2009 to September 2010, and the only inclusion criterion was the availability of sufficient blood volume to fill 2 tubes equally from a single collection of blood from dogs presented to a local veterinary hospital (Fregis, Arcueil, France). Blood was collected uniformly, in a volume of 2.5–3 mL per tube, into 2 3.5-mL tubes containing lithium heparin and gel (Venosafe, VF-054SAHL; Terumo Europe NV, Leuven, Belgium). One tube was within the expiration date, and the other tube had expired by 2, 4, 6, 7, 8, or 11 months.

Specimens were transported to the Vebiotel Laboratory, located minutes away from the veterinary hospital. To separate plasma from cellular elements, tubes

Table 1. Methods, imprecision, and values for canine plasma analytes measured in blood collected in non-expired tubes containing lithium heparin and gel.

Analyte	Method	Imprecision CV (%)	Median values (minimum/maximum)
Urea g/L	Enzymatic assay (urease; 37°C)	5.05	0.3 (< 0.07/7.3)
Creatinine mg/L	Kinetic Jaffé reaction (alkaline picrate solution; 37°C)	4.27	7 (5/206)
Total Protein g/L	Biuret assay (cupric ions; 37°C)	6.47	58 (37/78)
Albumin g/L	Bromcresol Green (37°C)	2.44	29 (23/36)
Total Bilirubin mg/L	Modified Diazo reaction (p-nitrobenzenediazonium salt; 37°C)	8.47	2 (1/51)
Cholesterol g/L	Enzymatic assay (cholesterol esterase and cholesterol oxidase; 37°C)	7.74	2.4 (0.9/5.7)
Triglycerides g/L	Enzymatic assay (lipase, glycerokinase, glycerol-3-phosphateoxidase; 37°C)	5.67	0.5 (0.2/2.4)
ALT U/L	Enzymatic assay (substrates: L-alanine and 2-oxoglutarate; 37°C)	6.98	60 (15/1215)
AST U/L	Enzymatic assay (substrates: L-aspartate and 2-oxoglutarate; 37°C)	5.64	27 (< 2/505)
ALP U/L	Enzymatic assay (substrate: p-nitrophenylphosphate; 37°C)	6.69	66 (11/5377)
GGT U/L	Enzymatic assay (substrates: L-γ-glutamyl-3-carboxy-4-nitroanilide and glycylglycine; 37°C)	7.03	5 (< 7/146)
Amylase U/L	Enzymatic assay (substrate: ethylidene-pNP-G7; 37°C)	8.28	721 (251/4315)
Lipase U/L	Enzymatic assay (substrate: 1.2-o-dylauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester; 37°C)	9.21	79 (12/3668)
Creatine Kinase U/L	Enzymatic assay (substrates: creatine phosphate and ADP; 37°C)	5.86	154 (39/5449)
LDH U/L	Enzymatic assay (substrates: L-alanine and NAD; 37°C)	4.59	66 (19/302)
Fructosamine μmol/L	Colorimetric assay (nitrotetrazolium blue; 37°C)	6.39	259 (169/394)
Magnesium mg/L	Colorimetric assay (sodium salt of xylidyl blue I; 37°C)	5.34	16 (9/28)
Calcium mg/L	Colorimetric assay (arsenazo III dye; 37°C)	4.20	103 (77/119)
Phosphates mg/L	Colorimetric assay (ammonium molybdate; 37°C)	4.20	40 (22/196)
Sodium mmol/L	ISE direct potentiometry (22°C)	3.78	146 (130/160)
Potassium mmol/L	ISE direct potentiometry (22°C)	3.01	3.9 (3.3/5.2)
Chloride mmol/L	ISE direct potentiometry (22°C)	4.54	107 (78/135)
Total CO_2 mmol/L	Enzymatic assay (substrate: phosphoenolpyruvate carboxylase; 37°C)	8.60	24 (13/39)

$n = 61$ dogs, except $n = 54$ for total CO_2 measurement.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CV, coefficient of variation; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; ISE, ion-selective electrode.

were centrifuged (Heraeus Megafuge 16 centrifuge; Thermo Scientific, Osterode, Germany) within 1 hour of specimen collection at 1500*g*, room temperature (average 22°C), for 6 minutes. Plasma color was inspected for hemolysis, lipemia, or icterus; if preset, specimens were excluded to avoid interference with the biochemical assays. Analyses of 23 analytes were performed immediately after plasma separation using a Konelab 20i chemistry analyzer (Thermo Scientific) with defined methods and performance (Table 1). Quality control was monitored 2–3 times per week (up to 50 analyses between control runs) using normal, high, or low control material (Konelab, Cergy Pontoise, France) depending on the analyte.

Statistical analysis

Differences between the logarithm of concentrations or activities obtained from specimens collected in expired and non-expired tubes were computed and analyzed using a linear model that included a constant with time post-expiration as a fixed factor; the expiration factor accounted for a non-constant effect of time of expiration on the measured concentrations or activities. When this factor was not significant, the nullity (value equal to 0) of the constant was investigated using a Fisher's test. The exponential function of this constant was the median variation expressed as a percentage of the concentrations or activities obtained from specimens collected in expired vs non-expired tubes. Significance was set at $P < .05$.

Results

Blood specimens were collected from 61 dogs of various age (6 months to 16 years), sex (male or female), disease status (healthy or sick), and state of consciousness (anesthetized or awake). Specimens were collected from 11, 8, 8, 10, 12, and 12 dogs into tubes that had expired by 2, 4, 6, 7, 8, and 11 months, respectively, and into non-expired tubes. Plasma specimens had no visible hemolysis, lipemia, or icterus. Total CO_2 concentration was not measured in the first 7 dogs owing to insufficient reagent.

For the majority of analytes, results from specimens collected in expired and non-expired tubes did not differ significantly (Table 2). Activities of alkaline

Table 2. Differences in values for canine plasma biochemical analytes between specimens collected into non-expired and expired tubes.

	Median (maximum/minimum) percent difference						
Months after expiration (*n**)	2 months (11)	4 months (8)	6 months (8)	7 months (10)	8 months (12)	11 months (12)	*P*
Urea	0 (33/0)	0 (7/−100)	0 (33/0)	0 (25/0)	0 (2/−20)	0 (25/0)	NS
Creatinine	0 (14/−3)	0 (11/−40)	0 (14/0)	0 (14/−13)	0 (20/−14)	0 (14/−17)	NS
Total Protein	0 (5/−4)	−1 (8/−24)	−1 (6/−6)	−2 (5/−10)	−1 (7/−7)	−2 (3/−9)	NS
Albumin	−3 (8/−12)	−3 (17/−38)	0 (6/−4)	−1 (10/−9)	0 (4/−3)	−2 (4/−7)	NS
Total Bilirubin	0 (10/0)	0 (20/0)	0 (0/−2)	0 (200/0)	0 (0/0)	0 (33/−100)	NS
Cholesterol	0 (10/−15)	0 (25/−14)	0 (4/−8)	0 (7/−7)	0 (12/−25)	0 (11/−20)	NS
Triglycerides	0 (11/−20)	0 (25/−50)	0 (17/0)	0 (0/−25)	0 (20/−33)	0 (20/−8)	NS
ALT	0 (2/−21)	0 (6/0)	0 (5/−6)	−1 (3/−7)	0 (4/−4)	0 (12/−5)	NS
AST	−1 (11/−11)	0 (50/−12)	0 (5/−16)	−1 (92/−27)	−1 (9/−11)	−7 (8/−52)	NS
ALP	1 (2/−6)	0 (7/−5)	0 (8/−2)	0 (6/−3)	0 (8/−4)	1 (18/−7)	.039
GGT	0 (25/−50)	−20 (25/−150)	20 (100/−17)	5 (100/−200)	18 (50/−50)	13 (125/−7)	NS
Amylase	0 (2/−6)	−1 (1/−11)	1 (6/−2)	1 (6/−2)	0 (1/−3)	1 (2/−3)	NS
Lipase	−2 (4/−10)	−10 (3/−53)	1 (4/−8)	−3 (13/−14)	−3 (15/−25)	−4 (19/−100)	.027
Creatine Kinase	2 (22/−7)	0 (12/−10)	−2 (12/−16)	−4 (10/−52)	0 (11/−14)	−3 (13/−56)	NS
LDH	9 (36/−3)	6 (70/−7)	−2 (47/−55)	0 (17/−57)	0 (18/−40)	−3 (82/−133)	NS
Fructosamine	0 (4/−2)	3 (8/−3)	0 (19/−3)	2 (13/−5)	2 (6/−18)	2 (11/−7)	.032
Magnesium	−7 (6/−15)	0 (6/−13)	0 (13/−11)	0 (12/−5)	−3 (8/−13)	0 (14/−25)	NS
Calcium	4 (7/−4)	−2 (3/−12)	1 (15/−8)	0 (2/−1)	0 (11/−7)	0 (8/−4)	NS
Phosphates	0 (7/−4)	−2 (3/−26)	0 (3/−7)	−1 (9/−38)	0 (7/−44)	0 (40/−8)	NS
Sodium	0 (1/0)	0 (1/−1)	0 (1/−1)	0 (1/0)	0 (1/−1)	0 (1/−1)	NS
Potassium	0 (7/−8)	0 (2/−3)	0 (3/−3)	0 (3/−3)	1 (3/−11)	0 (3/−3)	NS
Chloride	0 (1/−1)	0 (2/−1)	0 (1/−1)	0 (1/−1)	0 (0/−2)	0 (1/−1)	NS
Total CO_2	0 (0/−9)	0 (4/−8)	4 (12/−6)	0 (5/−24)	−4 (4/−53)	−3 (5/−15)	.032

*n indicates number of specimens compared, except for total CO_2 for which n = 4, 8, 8, 10, 12, and 12 for 2, 4, 6, 7, 8, 11 months, respectively.
ALP, alkaline phosphatase; ALT alanine aminotransferase; AST, aspartate aminotransferase; GGT; γ-glutamyl transferase; LDH, lactate dehydrogenase; NS, not significant.

phosphatase (ALP) and lipase and concentrations of fructosamine and total CO_2 were significantly different (P = .039, .027, .032, and .032, respectively) between the two tubes. The effect of using expired tubes on ALP and lipase activities and fructosamine and total CO_2 concentrations was not significantly different for different times post-expiration (Figure 1), and the median variations of (non-expired–expired)/non-expired were 1.3, −4.5, 1.7, and −2.6%, respectively. On close inspection of differences, 3 differences (Figures 1B and 1D) could have be considered as outliers according to Tukey's criterion of ± 3 interquartile range: 2 for lipase activity (4 and 11 months post-expiration) and 1 for total CO_2 concentration (8 months post-expiration). Other than the use of expired tubes, analytical or pre-analytical conditions could not be found to account for these differences. If these values were excluded, then the corresponding relative median differences were 2.1 and 0.0%, for lipase activity and total CO_2 concentration, respectively.

For ALP and lipase activities and fructosamine and total CO_2 concentrations, the largest difference was usually found when dogs had values within the reference intervals established by the laboratory (based on measurements of specimens collected in non-expired tubes) with the exception of total CO_2. In these circumstances, differences were 18% (11 U/L) for ALP at, −100% (15 U/L) for lipase, 19% (299 μmol/L) for

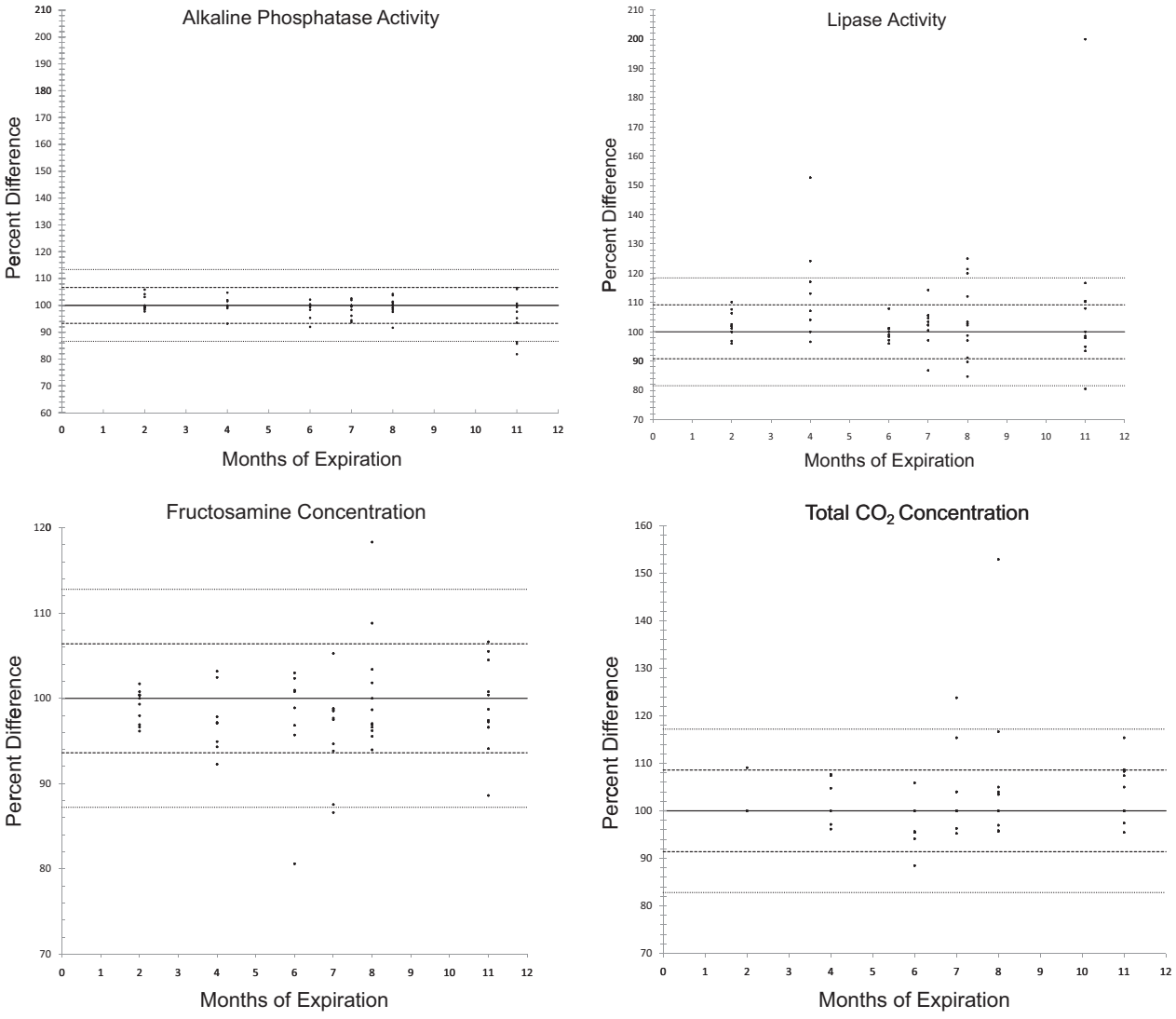

Figure 1. Individual proportional differences (%) between measurements obtained from specimens collected in non-expired and expired tubes containing lithium heparin and gel. Only analytes for which significant differences were found are shown. Dotted lines represent ± 1 and 2 SDs (expressed as coefficients of variation [%]).

Table 3. Differences in values for canine plasma analytes between specimens collected into non-expired and expired tubes when values for 4 analytes were outside the reference interval.

Analyte	n*	Median (minimum/maximum) percent difference	Minimum value	Maximum value	Reference interval
ALP U/L	21	0 (−4/2)	116	5377	< 100
Lipase U/L	8	−1 (−6/5)	424	3668	< 400
Fructosamine μmol/L	33	2 (−5/8)	169	394	260–340
Total CO2 mmol/L	25	0 (−53/12)	13	39	20–25

*Number of dogs with values outside reference interval.
ALP, alkaline phosphatase.

fructosamine, and −53% (17 mmol/L) for total CO_2 (Tables 2 and 3). Differences > 2 coefficient of variation of the respective method were 14% (at 22 and 56 U/L) and 18% (at 11 U/L) for ALP activity; −100% (at 15 U/L), −53% (at 93 U/L), −25% (at 20 U/L), −24% (at 62 U/L), −21% (at 42 U/L), −20% (at 70 U/L), and 19% (at 159 U/L) for lipase activity; −18% (at 262 μmol/L), 13% (at 336 μmol/L), and 19% (at 299 μmol/L) for fructosamine concentration; and −53% (at 17 mmol/L) and −24% (at 21 mmol/L) for total CO_2 concentration.

Discussion

For most analytes, accurately measurements could be obtained when specimens were collected into lithium heparin/gel tubes that were up to 11 months post-expiration, and measurement of the majority of the analytes in specimens collected into expired tubes did not change the classification of results in comparing them with reference intervals. Although significant differences were found for ALP and lipase activities and fructosamine and total CO_2 concentrations, differences did not increase with increasing time post-expiration and percent differences were low in most cases. For fructosamine and CO_2, however, observed differences may have lead to misclassification of the result and possible erroneous interpretations. The clinical relevance of the observed differences is difficult to assess. The only objective criterion may be the critical difference, which, unfortunately, has only been determined in dogs for urea, creatinine, total protein, albumin, glucose, and cholesterol concentrations and ALP, ALT, AST activities and which varied from 7.9% to 46.7%.[10]

The lack of differences found in this study may not be applicable to specimens processed > 1 hour after collection. A study of analysis of specimens stored for varying times in expired tubes and comparison with the reported 3-day stability of analytes in heparinized plasma (chloride and total CO_2 not included)[11] might be useful. If expired tubes are used inadvertently, processing and analysis of specimens within 1 hour are recommended. Additional studies are needed with larger numbers of specimens to evaluate possible effects on other analytes, wider ranges of values, collection into other anticoagulants, and other species.

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