Evaluation of an Automated Colorimetric Assay for the Measurement of Lipase Activity in Canine Sera

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ABSTRACT

An automated colorimetric method for determining lipase activity in canine sera was evaluated for precision, linearity and correlation to existing assay methods. The colorimetric method was a commercial reagent that used a series of enzymatic reactions based on the hydrolysis of 1,2 diglyceride by pancreatic lipase. Within-run and between-run coefficients of variation were <6.8% and <8.3%, respectively. Linearity was determined to be at least 1366 U/L. Canine serum lipase concentrations attained using the colorimetric method were compared to both titrimetric and dry-film methods for measuring serum lipase activity, resulting in significant ($P \leq 0.05$) correlation coefficients of 0.92 and 0.77, respectively. Canine serum lipase concentrations measured using the colorimetric assay on 2 different automated analyzers had a significant ($P \leq 0.05$) correlation coefficient of 0.92. A laboratory reference range using serum samples from 56 healthy dogs (0–561 U/L) was established. There were no significant ($P \leq 0.05$) differences in mean serum lipase concentrations comparing male and female dogs or comparing young dogs (≤3 y) to mature (4–7 y) and older (>7 y) dogs using this assay. It was concluded that the automated colorimetric assay was a reliable indicator of canine serum lipase activity and offered several advantages, including small sample volume and short analysis time.

RÉSUMÉ

Une méthode colorimétrique et automatisée pour déterminer l'activité de la lipase dans le sérum de chien a été évaluée pour sa précision, sa relation linéaire et son degré de corrélation avec des méthodes existantes. La méthode colorimétrique consiste en un réactif commercial qui utilise une série de réactions enzymatiques basées sur l'hydrolyse de diglycéride par la lipase pancréatique. Les coefficients de variation intra-essai et inter-essai étaient respectivement inférieurs à 6,8% et 8,3%. La linéarité a été déterminée comme étant d'au moins 1,366 U/L. Les concentrations de lipase sérique canine obtenues par la méthode colorimétrique ont été comparées à celles obtenues par les méthodes de titrage et du film sec, et les résultats indiquent des coefficients de corrélation significatifs ($P \leq 0,05$) de 0,92 et 0,77. Les résultats obtenus par la méthode colorimétrique à l'aide de deux appareils différents avaient un coefficient de corrélation significatif ($P \leq 0,05$) de 0,92. À l'aide d'échantillons de sérum prélevés chez 56 chiens en santé, des valeurs de référence (0–561 U/L) ont été établies. Avec l'épreuve colorimétrique, aucune différence significative dans les concentrations sériques moyennes de lipase n'a été notée entre les mâles et les femelles, entre des chiens âgés de ≤3 ans, de 4 à 7 ans ou de plus de 7 ans. Cette méthode apparaît comme un bon indicateur de l'activité de la lipase sérique chez le chien et offre comme avantages le besoin d'avoir une petite quantité d'échantillon et un temps d'analyse court.

(Traduit par docteur Serge Messier)

INTRODUCTION

Measurement of serum lipase activity is utilized for the diagnosis of pancreatic acinar cell injury in dogs (1,2). Many of the current methods of lipase analysis rely on titrimetric procedures, which are labour intensive, time consuming, and require large sample volumes (3). Recently introduced alternatives to traditional titrimetric procedures include dry-film, turbidimetric and colorimetric methods. Reagent and instrumentation costs associated with dry film techniques may make this choice prohibitive for some laboratories (4). Both turbidimetric and colorimetric methods can have difficulties caused by interfering substances and reagent instability (5,6).

A new colorimetric method for lipase determination in human sera (7) has recently become commercially available. This method utilizes a colorimetric reaction linked to a series of enzymatic processes based on the hydrolysis of 1,2 diglyceride by pancreatic lipase. This procedure offers the advantage of specifically measuring lipase in human sera, which is of pancreatic origin, since hepatic lipase and lipoprotein lipase fail to increase measured human serum lipase activity using this assay (8). To our knowledge, it is not known what effect, if any, hepatic lipase or lipoprotein lipase may have on canine serum

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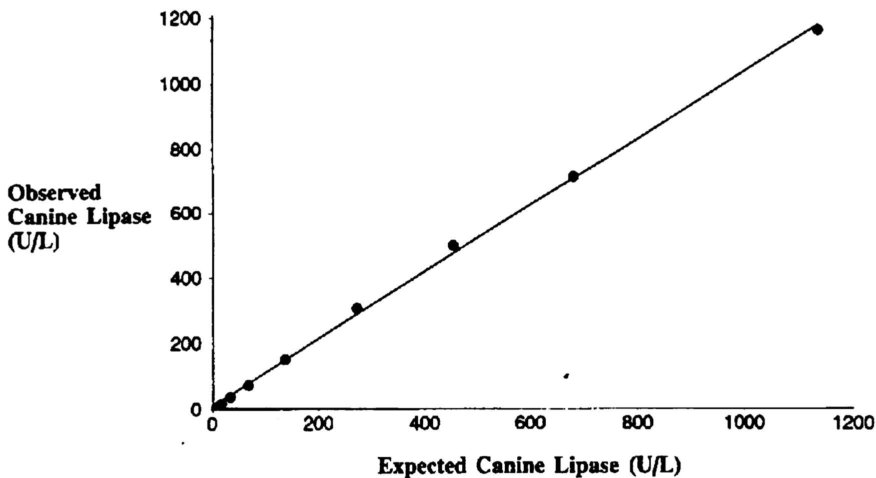

Figure 1. Linearity plot of saline dilutions of a serum sample with a high lipase concentration (1366 U/L). Observed canine serum lipase concentrations correlate closely (within 10%) with expected concentrations.

lipase activity. However, it is recognized that conditions other than pancreatitis are associated with increased serum lipase concentrations in the dog, including renal disease (2,9), hepatic disease (2), and corticosteroid treatment (10). The specificity of the colorimetric assay for pancreatic lipase in human sera has been attributed to the use of 1,2 diglyceride as the substrate, and both deoxycholate and co-lipase as specific enhancers of pancreatic lipase activity (1,11). Human pancreatic lipase has been previously found to have similarities in amino acid composition, immunogenicity and glycoprotein molecular weight to other mammalian species, including the dog (12). This study was designed to evaluate the precision, linearity and intermethod agreement of this assay for canine sera using automated analyzer technology. An additional goal was to establish a reference range for serum lipase activity in healthy dogs using this method.

MATERIALS AND METHODS

SAMPLE COLLECTION

Canine serum samples were chosen from those submitted to the diagnostic laboratories of the Atlantic Veterinary College and the Western College of Veterinary Medicine. Samples for determination of the laboratory reference range were analyzed without prior freezing. Samples for correlation studies were either analyzed without prior freezing or were stored at −29°C for less than 2 mo before analysis; canine serum lipase activity is considered to be stable when frozen (1). In order to evaluate a wide range of serum lipase concentrations, the serum samples were chosen from both animals judged as normal and those with various clinical diagnoses, including pancreatitis. The animals judged as normal were categorized as such on the basis of physical examination findings and lack of appreciable serum biochemical profile and complete blood count abnormalities.

MEASUREMENT OF SERUM LIPASE ACTIVITY

The majority of the colorimetric lipase assays were performed on one automated analyzer (DACOS, Coulter Electronics, Hialeah, Florida, USA), and data presented are from this analyzer, unless otherwise stated. Additional precision data for both within-run and between-run analyses were attained using a second automated analyzer (Hitachi 911, Boehringer Mannheim Corp, Laval, Québec). The lipase activity was determined using a commercially available reagent (DCL Lipase, Diagnostic Chemicals Ltd, Charlottetown, PEI), with assay conditions modified by the Atlantic Veterinary College Diagnostic Services Laboratory for use on both automated analyzers.

Modified conditions for analysis on the first analyzer (DACOS) included the following: 37°C temperature, 4 uL sample volume, 20 uL diluent volume, 250 uL reagent 1 volume (includes 1,2 diglyceride), and 80 uL reagent 2 volume (includes deoxycholate). Other conditions included a one channel analysis, a 550 nm wavelength and a linear reaction type. A two point calibration using the standard provided with the reagent kit was performed. The method of standard curve calibration was regression with a reagent blank. Automated analysis utilizing the second automated analyzer (Hitachi 911) used the following conditions: 37°C, 5 uL sample volume, 300 uL reagent 1 volume, and 100 uL reagent 2 volume. Other conditions included a rate-A reaction, bichromatic wavelengths of 546 nm (main) and 660 nm (secondary), and a 2 point linear calibration.

For intermethod comparison of the serum lipase concentrations, canine sera were also analyzed using a manual titrimetric method (3). The titrimetric method utilized a commercial reagent (Sigma Chemical Co, St. Louis, Missouri, USA) with olive oil as the substrate and bovine-based quality control sera (Sigma 2E, Enzyme Control, Sigma Diagnostics, St. Louis, Missouri, USA) as an external control. A second intermethod comparison was performed on canine serum samples using the colorimetric method and a dry-film technique. This latter method used a dry-film analyzer (Vet Test 8008, Idexx Laboratories Inc, Westbrook, Maine, USA) and licensed dry-film slides and controls (Eastman Kodak, Rochester, New York, USA).

ASSAY EVALUATION

To evaluate the precision of the colorimetric method for measuring serum lipase concentrations, within-run and between-run (day-to-day) coefficient of variation (CV) calculations were performed. Three samples were analyzed 10 times on the first analyzer (DACOS) to attain data for the within-run calculations. Two of the 3 samples were control sera, consisting of a bovine albumin-based control sera (Sigma 2E, Enzyme Control, Sigma Diagnostics) with a mean lipase concentration of 42 U/L and a human-based control sera (DCTrol Level 2, Diagnostic Chemicals Ltd) with a mean lipase concentration of 643 U/L. The third sample utilized for

within-run precision was a serum sample from a healthy dog with an intermediate lipase concentration of 83 U/L. This value was determined by calculating the mean lipase concentration attained from the 10 repeat determinations of lipase concentrations utilizing the colorimetric assay. The 2 control sera used for the within-run calculations were also analyzed 10 times over a period of 10 d to obtain between-run precision data. In addition, 3 canine serum samples with mean lipase concentrations of 54 U/L, 103 U/L and 644 U/L were analyzed on the second analyzer (Hitachi 911) for both within-run (10 times) and between-run (daily for 10 d) data. These concentrations were determined by calculating the mean lipase concentration attained from the 10 repeat determinations of lipase concentrations using the colorimetric assay.

Linearity of the colorimetric procedure was evaluated by measuring the lipase concentrations of a series of saline dilutions of a canine serum sample with a high lipase concentration of 1366 U/L. Percentages for the individual dilutions (with expected results in brackets) were as follows: 83% (1138 U/L), 50% (683 U/L), 33% (455 U/L) and 20% (273 U/L). The remaining saline dilutions were performed in a serial fashion from the 20% sample (with expected results in brackets) as follows: 10% (137 U/L), 5% (68 U/L), 2.5% (34 U/L), 1.25% (17 U/L), and 0.635% (8.5 U/L).

Intermethod comparison of canine serum lipase concentrations were performed utilizing 36 serum samples which were assayed using both the colorimetric method and the titrimetric method. Twenty-three samples of canine sera were analyzed using both the colorimetric assay and the dry-film technique for the 2nd intermethod comparison. Twenty-two canine serum samples were analyzed using the colorimetric method on both automated analyzers for a comparison between the instruments.

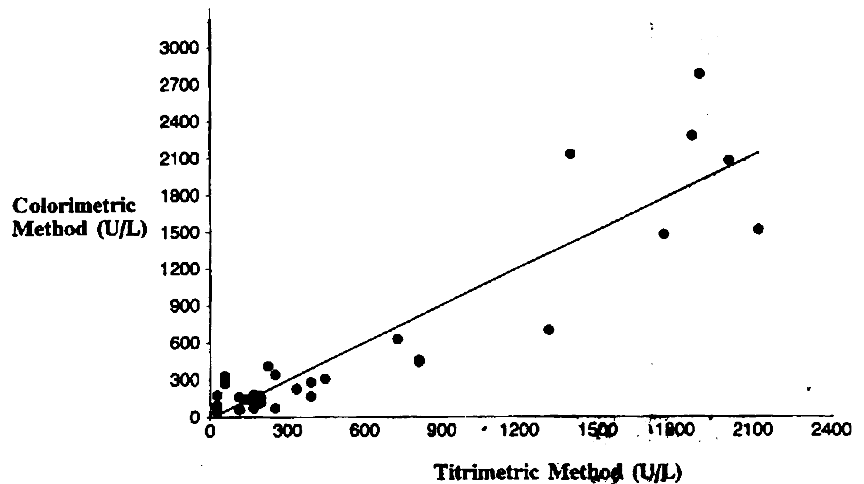

Figure 2. Regression analysis plot comparing lipase concentrations (U/L) in 36 samples of canine sera obtained using both the colorimetric method and the titrimetric method. The regression equation derived was y = −9.0 + 1.01x and the significant ($P \leq 0.05$) correlation coefficient *r* was 0.92.

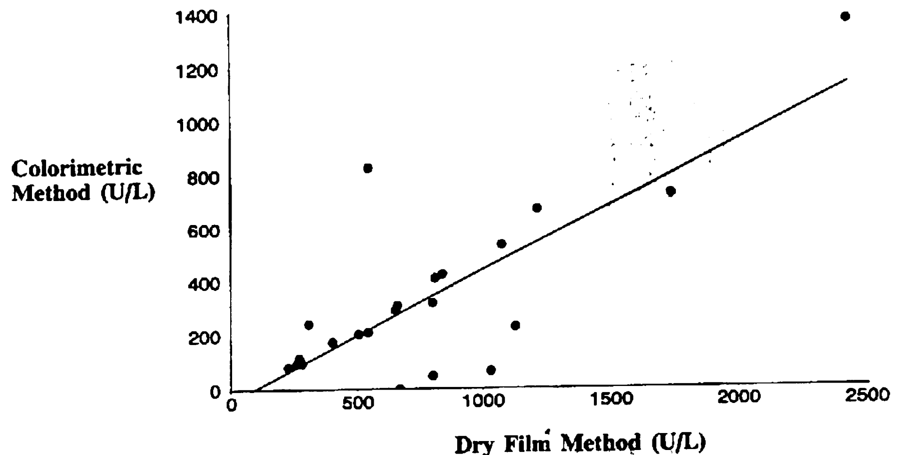

Figure 3. Regression analysis plot comparing lipase concentrations (U/L) in 23 samples of canine sera obtained using both the colorimetric method and the dry film method. The regression equation derived was y= −33.9 + 0.479x and the significant ($P \leq 0.05$) correlation coefficient *r* was 0.77.

STATISTICAL ANALYSIS

A computer software program (Minitab Statistical Software, Standard Version 9.1, Minitab Computer Software Inc, State College, Pennsylvania, USA) was used for statistical calculations. All tests were performed at the ($P \leq 0.05$) significance level. Linear regression analysis was performed for the comparison between serum lipase concentrations using the colorimetric assay and titrimetric assay, as well as for the comparison between the colorimetric assay and the dry-film method. A linear regression analysis was also performed for the comparison between measurements obtained using the colorimetric method on both automated analyzers. The reference range for canine serum lipase concentration was determined by calculating the mean +/− 2 standard deviations for 56 normal dogs. Canine serum lipase concentrations determined in normal dogs with the colorimetric assay were further divided into subsets based on sex (21 males and 35 females) and age (23 young dogs [≤3 y] 18 mature dogs [4–7 y] and 15 older dogs [>7 y]). A student's *t*-test (two-sided interval) and a one-way analysis of variance, respectively, were used to determine if significant differences based on sex or age existed for the mean lipase activities of these subgroups in canine sera.

RESULTS

The results of the precision study are summarized in Table I. Coefficients

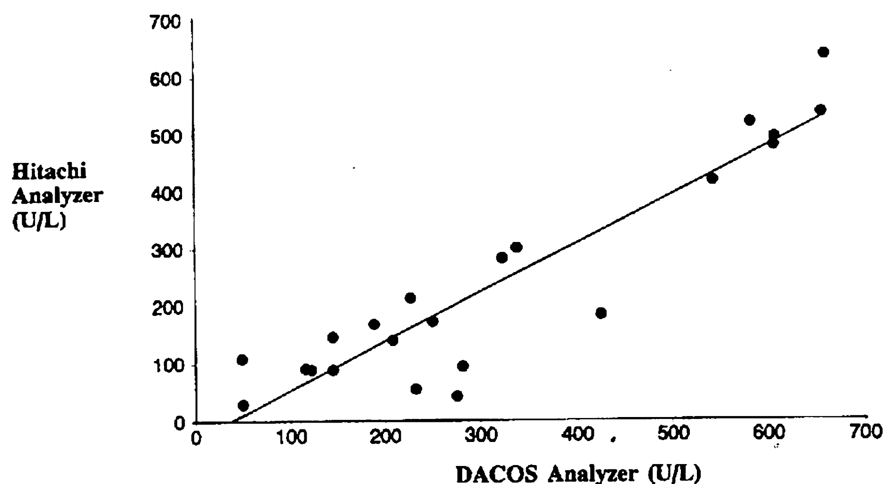

Figure 4. Regression analysis plot comparing lipase concentrations (U/L) in 22 samples of canine sera obtained with the colorimetric method on the DACOS (Coulter Electronics, Hialeah, Florida) and the Hitachi 911 (Boehringer Mannheim, Laval, Québec) automated analyzers. The regression equation was $y = -31.3 + 0.846x$ and the significant ($P \leq 0.05$) correlation coefficient r was 0.92.

TABLE I. Precision data for the colorimetric assay performed with control sera and canine sera on two automated analyzers

Sample	Control serum (bovine albumin) based)	Control serum (human sera-based)	Canine serum 1	Canine serum 2	Canine serum 3	Canine serum 4
Analyzer	DACOS[a]	DACOS[a]	DACOS[a]	Hitachi[b]	Hitachi[b]	Hitachi[b]
Within-run:						
Mean lipase concentration (U/L)	42	643	83	54	103	644
CV%[c]	6.7	0.7	4.6	0.9	0.7	0.3
n	10	10	10	10	10	10
Between-run						
Mean lipase concentration (U/L)	45	656	—	54	102	644
CV%[c]	8.2	3.5	—	4.1	3.3	4.6
n	10	10	—	10	10	10

[a] DACOS automated analyzer, Coulter Electronics, Hialeah, Florida, USA
[b] Hitachi 911, Boehringer Mannheim Corp., Laval, Québec
[c] Coefficient of variation

of variation for within-run and for between-run analyses were less than 8.3% in all calculations. Since dilutions of a canine serum sample with a high lipase concentration (1366 U/L) gave observed results within 10% of expected (Figure 1), the colorimetric assay was considered to be linear to at least 1366 U/L. The relationship between the serum lipase concentrations using the colorimetric assay and the titrimetric method is shown in Figure 2. The comparison between lipase concentrations determined with the colorimetric method and the dry film technique is illustrated in Figure 3. In the first comparison, the correlation coefficient r was 0.92 and was significant. A significant correlation coefficient (0.77) was also seen for the comparison between the colorimetric assay and the dry film assay. The significant correlation result (r = 0.92) for the comparison between lipase concentrations using the colorimetric assay on both automated analyzers is shown in Figure 4.

Serum lipase concentrations in 56 healthy dogs were normally distributed and ranged from 0–779 U/L. The laboratory reference range was determined to be 275 +/− 286 (0–561 U/L). No significant differences were found between mean serum lipase concentrations of male (mean: 266 U/L) and female (mean: 292 U/L) dogs or between young (mean: 284 U/L), mature (mean: 282 U/L) and older (mean: 271 U/L) dogs.

DISCUSSION

The automated colorimetric assay evaluated in this study was determined to be a reliable indicator of serum lipase activity in the dog. The precision of the assay is good, with coefficients of variation of less than 6.8% for within-run and less than 8.3% for between-run (day-to-day) analysis. This is comparable to values reported for similar automated procedures (11).

Linearity as determined by our evaluation (1366 U/L) is greater than twice the upper limit of the reference range (561 U/L). This is an adequate value for linearity, as dilutions will only have to be made for patient samples with serum lipase concentrations greater than twice the upper limit of the reference range. True linearity may indeed be higher than 1366 U/L, but we were limited in the availability of canine serum samples with extremely high lipase concentrations to utilize in linearity evaluation.

Correlation results comparing canine serum lipase concentrations attained with the colorimetric assay to the titrimetric assay were excellent and the correlation to the dry-film assay was also acceptable. A moderate degree of scatter was seen in the higher concentration data points in the plot of the comparison to the titrimetric method (Figure 2). This dispersion may be partly attributed to analytical variance associated with manual dilutions required to analyze these samples. A good correlation (r = 0.92) was also achieved for the comparison between canine serum lipase concentrations using the colorimetric assay on 2 different automated analyzers. This correlation plot (Figure 4) has an acceptable degree of scatter, but there are also 4 data points for which the DACOS analyzer results were higher than those from the Hitachi 911. These data points seen well below the regression line on Figure 4 may represent samples in which an interfering substance could be causing a greater interference with one analyzer than with the other. Such interferences could include administered drugs or

endogenous substances (hemoglobin, etc) that were not appreciated at the time of sample analysis.

This study supports and expands upon data recently reported for the evaluation of a similar colorimetric assay for canine serum lipase using a different automated analyzer (13). Compared to this earlier study, the present study attained similar results for assay linearity and correlation of lipase concentrations to a titrimetric method. The reference range for canine serum lipase concentrations determined in the present evaluation (0–561 U/L) also agreed closely with the 1 reported in the earlier study (90–527 U/L)(13). Information provided in the present study which was unavailable previously includes the precision data, the correlation of lipase concentrations measured using the same method on 2 automated analyzers, and the correlation of lipase concentrations comparing the colorimetric method to a dry film method.

This new colorimetric procedure has several advantages. These include a small sample size (4 uL), a short analysis time (<5 min), and easy adaptability to automated analysis using various analyzers. This procedure can also be performed manually using a spectrophotometer. One disadvantage of the colorimetric method is that it is a 2-reagent system requiring extra space on analyzer reagent trays. In conclusion, the automated colorimetric assay is a reliable indicator of canine serum lipase concentration and offers the advantages of short analysis time and small sample size with good precision and linearity.

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