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Original article

The use of semi-automated methodology does not interfere significantly in the activity measurement of the urinary gamma-glutamyltransferase in dogs

A utilização da metodologia semiautomática não interfere significativamente na mensuração da atividade da gama glutamil transferase urinária em cães

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A R T I C L E I N F O

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A B S T R A C T

The urinary gamma-glutamyltransferase (GGTu) is a precocious indicator of renal lesion and the gold standard for the measurement of its activity is the automated method, although semi-automation is often utilized and studies relating this methodology to the occurrence and intensity of analytical errors are still scarce. Therefore, this work aimed to calculate the systematic and random errors in the determination of the GGTu activity in dogs with the use of the semi-automated method and evaluate if that methodology statistically differs from the automated method. 49 dog urine samples were collected through cystocentesis and centrifuged for separation of the supernatant, which was employed for the measurement of the GGTu activity by automated (reference) and semi-automated methods. Linear regression and Pearson correlation (r) tests were employed for the establishment of the systematic error. The random error was calculated according to Westgard; Hunt (1973). Lin's concordance correlation coefficient was employed to evaluate the presence of concordance between automated and semi-automated techniques. In the analysis of results, a constant error of + 9.51 UI/L (a = 9.5118), a proportional error of – 9.37% (b=0.9063) and a random error of 9.91% was observed when the semi-automated methodology was employed. The determination (R2) and Lin coefficients were, respectively, 0.9859 with p<0.0001 and 0.9912, suggesting a great similarity and almost perfect concordance between the two methods. Therefore, the data verified that the semi-automation does not interfere significantly in the measurement of the GGTu activity within the minimum and maximum values observed in the study.

Palavras-chave:
Erro sistemático
Erro randômico
Cão
Automação

R E S U M O

A gama glutamil transferase urinária (GGTu) é um biomarcador precoce de lesão renal e o padrão ouro para mensuração da sua atividade é o método automatizado, apesar disto, a semiautomação é frequentemente utilizada e são escassos os trabalhos que relacionam essa metodologia à ocorrência e intensidade de erros analíticos. Sendo assim, este trabalho teve como objetivo calcular os erros sistemático e randômico na determinação da atividade de GGTu de cães a partir do uso do método semiautomatizado e avaliar se essa metodologia difere estatisticamente do método automático. Coletou-se 49 amostras de urina de cães por cistocentese, as quais foram centrifugadas para separação do sobrenadante, que foi utilizado para a mensuração da atividade da GGTu pelos métodos automático, considerado como referência, e semiautomático. Os testes de regressão linear e correlação de Pearson (r) foram utilizados para o estabelecimento do erro sistemático. O erro randômico foi calculado de acordo com Westgard; Hunt (1973). Para avaliar a presença de concordância entre as técnicas automática e semiautomática foi empregado o coeficiente de concordância de Lin. Na análise dos resultados observou-se a presença de erro constante de + 9,51 UI/L (a = 9,5118), erro proporcional de - 9,37% (b=0,9063) e erro randômico de 9,91% quando a metodologia semiautomática foi utilizada. Os coeficientes de determinação (R2) e de Lin

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calculados foram, respectivamente, 0,9859 com p<0,0001 e 0,9912, indicando alta semelhança e concordância quase perfeita entre os métodos analisados. Dessa maneira, esses dados mostram que a semiautomação não interfere significativamente na mensuração da atividade da GGTu dentro dos valores mínimo e máximo observados no trabalho.

INTRODUCTION

The gamma-glutamyltransferase is an enzyme that is primarily located in the cells of the loop of Henle and in the proximal convoluted tubules of the nephrons (MELO et al., 2006), possessing antioxidant action and participating in the homeostasis of glutathione and in the transport of amino acids through the cell membranes (YESIL et al., 2014*)*. When measured in urine, this enzyme is a precocious biomarker of renal tubular lesions and precedes alterations in urinary density, serum biochemistry and in the histopathological examination of the patients (CRIVELLENTI et. al., 2014; GRAUER et al., 1994).

The enzymatic activity of the urinary GGT (GGTu) can be determined by automated and semi-automated methods (KOVARIKOVA, 2015). Automated techniques are considered the gold standard test for biochemical analyses, since they provide greater reliability and safety with the minimization of repeatability errors and individual variation between tests, besides the greater quickness to provide test results and the decrease in residue generation (CAMPANA; OPLUSTIL, 2011). Conversely, semi-automated techniques possess lower cost and greater accessibility and, for this reason, they are widely employed in several veterinary laboratories through the country. Semi-automation, however, elevates the percentage of errors due to possible failures in the calibration and variation among operators, besides requiring more time and volume for the processing of the samples.

In this perspective, studies comparing different measurement techniques of the same analyte are often performed to determine the degree of error expected based on the development analysis of the method. This performance evaluation takes into account criteria such as inaccuracy and imprecision, obtained through the calculation of analytical errors (JENSEN; KJELGAAD-HANSEN, 2006).

Methodological errors in laboratory analysis might occur due to several reasons, and compromise the results in distinct manners. The analytical error is obtained from the sum of the random and systematic errors (KOCH; PETERS, 1999). The imprecision in a test result can be evaluated with the determination of the random error (WESTGARD; HUNT, 1973). The systematic error is classified as proportional and constant and consists in the distance between the values obtained by the evaluating equipment and the correct value of the analyte, verified by the reference method (JENSEN; KJELGAAD-HANSEN, 2006).

The constant error is defined as systematic deviations estimated from the average difference between the two methods and is present when the value of the intercept (*a*) differs from zero (*a-0)*. When existing, the constant error indicates a decrease equivalent to its magnitude, in the specificity of the employed technique. The proportional error exists if the inclination (*b*) is different from one (b-1), and demonstrates that the difference between the two methods is related to the level of the measurements, signalizing that the calibration and programming procedures of the equipment need to be readjusted (JENSEN; KJELGAAD-HANSEN, 2006; WESTGARD; HUNT, 1973).

Since semi-automation is still often employed in several laboratories of clinical analyses, and that studies referring the occurrence and intensity of analytical errors in the measurement of the activity of the GGTu in this type of equipment, this work aimed to calculate the systematic and random errors in the determination of the activity of the GGTu from dogs based on the semi-automated method, as well as to evaluate if the methodology statistically differs from the automated method.

MATERIAL AND METHODS

Following the criteria by Bellamy; Olexson (2000), and based on the rate of change (JENSEN; KJELGAARD-HANSEN, 2006) for the determination of the size of the sampling group in comparison studies between methods, 49 dog urine samples from animals of different age and sex were used, belonging to a routine of clinical analyses in a laboratory of Clinical Pathology. Samples with normal and extreme values of GGTu activity were included in the experiment to provide a greater representation of the working range of the analyzed methods (JENSEN; KJELGAARD-HANSEN, 2006).

All urine samples were collected via cystocentesis, and the samples that presented active sediment or coloring alteration were discarded (CHEW; DIBARTOLA; SCHENCK, 2012). After centrifugation for 5 minutes at 1600 rpm, the supernatant was immediately used for the measurement of the activity of the urinary GGT, performed simultaneously by the automated equipment COBAS C111®, with Roche® reagent kits (USA), and by the semi-automated equipment Bioplus®, with Analisa® reagent kits (Brazil). Rules and instructions were employed according to the indications of the manufacturers of the equipment and reagents. For the decrease of the individual variation between tests, the measuring procedures obtained by the semi-automated technique were executed by the same professional. The quality control with control serum was daily performed

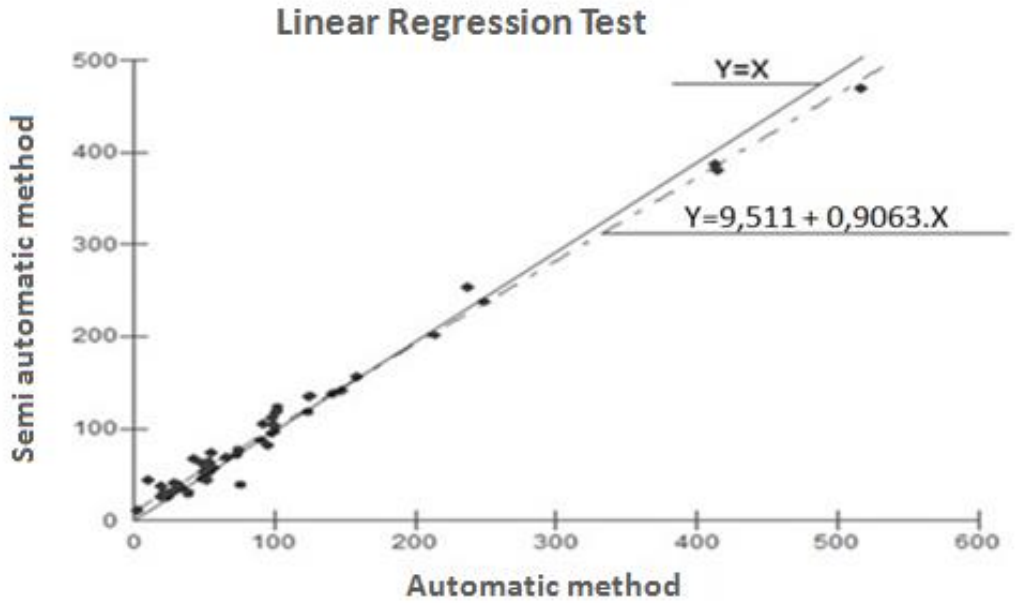
in both equipment. The values obtained in the automated method were used as reference for the analyses of the results (WESTGARD; HUNT, 1973). The linear regression test was used for the establishment of the systematic error, and the Pearson correlation (r) was employed for the validation of its results (WESTGARD; HUNT, 1973). The random error was calculated according to Westgard; Hunt (1973). The minimum and maximum values, as well as the median, were also calculated for both measuring methods of the GGTu, and the paired t test was used to evaluate the difference between these groups. The Bland-Altman plot was used to judge the acceptability of the tested methodology based on the imprecision of both methods (BLAND; ALTMAN, 1986). Lin's concordance correlation coefficient was employed in order to evaluate the presence of concordance between the two techniques (LIN, 1989), obtained through the digital calculation available in the website services.niwa.co.nz/services/statistical/concordance.
The statistical software employed in the experiment was the BioStat.

RESULTS AND DISCUSSION

The analysis of the results of the present study allowed to observe the presence of a constant error of + 9.51 UI/L (a = 9.5118) and a proportional error of – 9.37% (b=0.9063) when the semi-automated methodology was employed. The coefficient of determination (R2) calculated by the linear regression test was 0.9859 with p<0.0001. The closer to one (1.0) is the value of R2, the greater is the correlation between the analyzed methods (JENSEN; KJELGAAD-HANSEN, 2006). The Pearson correlation test resulted in a correlation coefficient (r) of 0.99, with p<0.001, demonstrating significance and validating the data of the linear regression.

By analyzing the graphic (Figure 1) it is possible to note that the measure of the value of the GGTu activity increases the regression line and deviates from the perfect regression (a=0; b=1). This variation occurred as a consequence of a discrete proportional error, and represents an addition in the difference between the results of the GGTu obtained by the automated and semi-automated methods in clinical pictures in which the activity of this enzyme is increased.

Figure 1 – Systematic error in the measurement of the GGTu (UI/L) in dogs by semi-automated method.



The X axis represents the automated method, considered as reference, and the Y axis represents the semi-automated method. The continuous line Y=X corresponds to the regression of perfectly symmetrical tests. The dotted line refers to the results of the analyzed samples, with intercept (a) equal to 9.511 and slope (b) of 0.9063.
The distance observed between the regression line and the perfect regression was more notorious from 102 UI/L, approximately. Such information, summed to the low degrees of constant and proportional errors found in the study, attests that the systematic error in the semi-automated methodology is not able to modify its results to the level of compromising the identification of tubular lesions and, therefore, it does not interfere in the clinical diagnostic. Nevertheless, measurements above 102 UI/L should be analyzed with caution.

The random error values observed for the automated and semi-automated equipment in the experiment were, respectively, 4.66% and 9.91%. The difference of the error between the techniques is expected and might occur due to the instability of the instruments employed in the semi-automated method, variation in the room temperature and individual variation in technical procedures such as pipetting and preparation of reagents (LUMSDEN, 2000). Random errors can be accepted since all methods routinely employed in

laboratories possess some degree of imprecision (WESTGARD; HUNT, 1973).

Based on the analysis of the Table (Table 1), it may be observed that the mean and median values of the semi-automated test were similar to those of the automated method. Minimum and maximum values stipulate the interval within which the statistical analysis is valid. Therefore, it is important to highlight that the results obtained in the present study are not representative of measurements of the GGTu activity higher than 517.6 UI/L or lower than 2.4 UI/L.

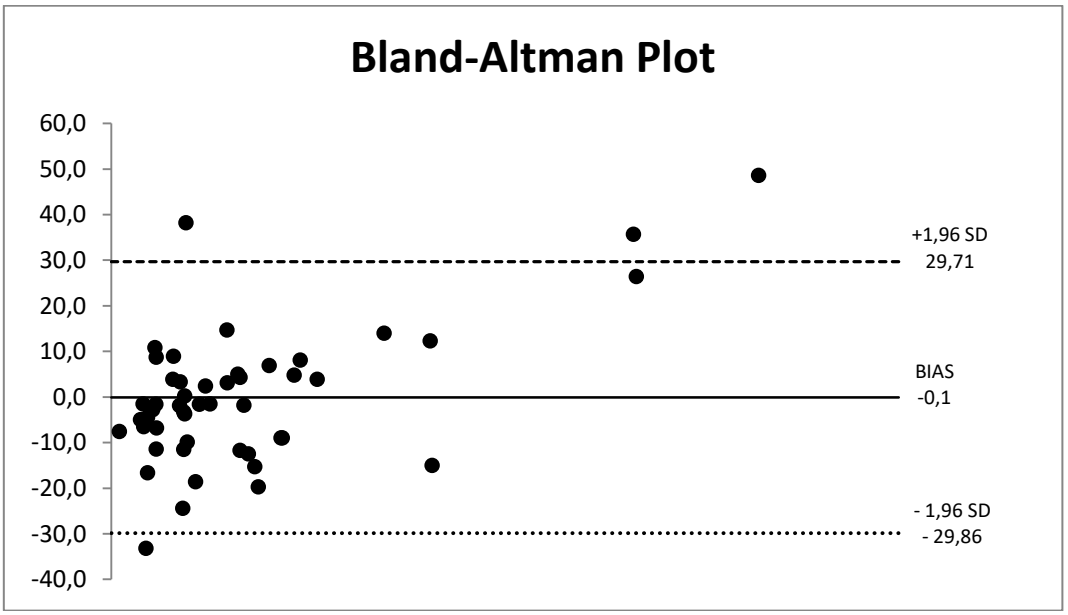
The paired t test resulted in values of (t) = 0.0348 and (p) = 0.9724, in a confidence interval of 95%, demonstrating a good similarity between the two tested methodologies. The methodology initially proposed by Bland; Altman (1986) to evaluate the concordance between two variables (X and Y) is based on a graphic visualization from a graph of the dispersion between the difference of such variables (X - Y) and the mean of these same variables (X + Y)/2 (HIRAKATA; CAMEY, 2009). In the Bland-Altman plot (Figure 2) it may be seen that the error, characterized by the dispersion of the difference dots around the mean is small and most of the plotted values are close to the mean, with few outliers. The bias obtained a value of -0.1. This parameter is given by the mean of the differences and corresponds to how much they deviate from the zero value.

Table 1 – Mean, median, minimum value, maximum value and random error calculated based on the statistical analysis of the results of the automated and semi-automated methods employed in the experiment for the measurement of the GGTu activity.

Parameters	Methods	
	Automated	Semiautomated
Mean	100.7	100.8
Median	66.4	68.0
Min Value	2.4	10.0
Max Value	517.6	469.0
*Mean CV%	4.66	9.91

*Random error (JENSEN and KJELGAAD-HANSEN, 2006).

Figure 2 – Correlation between the automated and semi-automated methods for the measurement of the GGTu (UI/L) in dogs.



Lin's coefficient was 0.9912, demonstrating an almost perfect concordance between the employed techniques. The data suggest that the semi-automated method does not interfere significantly in the measurement of the GGTu activity within the minimum and maximum values observed in the study.

CONCLUSION

Discrete analytical errors are present in the measurement of GGTu activity though semi-automated method. However, this methodology does not statistically differ from the reference methodology and can be employed in laboratory routine.

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