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 glutamyl transferase urinária em cães

Murilo Catelani Ferraz¹*, Marcel Capelini Sartoretto¹, Gustavo Gomes de Oliveira¹, Mikaelle de Oliveira Castilho¹, Tamires Ramborger Antunes¹, Alda Izabel de Souza¹

¹ Universidade Federal do Mato Grosso do Sul (UFMS), Campo Grande/MS, Brasil.
INTRODUCTION

The gamma-glutamyltransferase (GGTu) 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 in the transport of amino acids through the cell membranes (YESIL et al., 2014). When measured in urine, this enzyme is a precious 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; KJELGAARD-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 (KOCHE; 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; KJELGAARD-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, signaling that the calibration and programming procedures of the equipment need to be readjusted (JENSEN; KJELGAARD-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

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

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.
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.

| Parameters | Automated | Semi-automated |
|------------|-----------|----------------|
| Mean       | 100.7     | 100.8          |
| Median     | 66.4      | 68.0           |
| Min Value  | 2.4       | 10.0           |
| Max Value  | 517.6     | 469.0          |

*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.
CRIVELLENTI, L. Z. et al. False positivity of gamma-glutamyl transpeptidase measurement in urine. Renal Failure, v. 36, n. 4. p. 581-584, 2014.

GRAUER, G. F. et al. Effects of dietary protein conditioning on gentamicin-induced nephrotoxicosis in healthy male dogs. American Journal of Veterinary Research, v. 55, p. 90-97, 1994.

HIRAKATA, V. N.; CAMEY, S. A. Análise de concordância entre métodos de Bland-Altman. Revista do Hospital das Clínicas e da Faculdade de Medicina, v. 29, n.3, 2009.

JENSEN, A. L.; KJELGAARD-HANSEN, M. Method comparison in the clinical laboratory. Veterinary clinical pathology, v. 35, n. 3, p. 276-286, 2006.

KOH, D.D.; PETERS T. Selection and evaluation of methods. In: BURTIS, C. A.; ASHWOOD, E. R. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: Saunders Company, 1999. p. 320-335.

KOVARIKOVA, S. Urinary Biomarkes Of Renal Funcion In Dogs And Cats: a review. Veterinarndi Medicina, v. 60, n. 11, p. 589-602, 2015.

LIN, L. I. A. Concordance Correlation Coefficient to Evaluate Reproducibility. International Biometric Society, v. 45, n. 1, p. 255-268, 1989.

LUMSDEN, J. H. Laboratory test method validation. Revue de Médecine Vétérinaire, v.157, n.7, p. 623-630, 2000.

MELO, D. A. S. et al. Evaluation of renal enzymuria and cellular excretion as a marker of acute nephrotoxicity due to an overdoses of paracetamol in Wistar rats. Clinica Chimica Acta, v. 373, p. 88-91, 2006.

WESTGARD, J. O.; HUNT, M. R. Use and interpretation of Common Statistical tests in Method-Comparison Studies. Clinical Chemistry, v. 19, n. 1. p. 49-57, 1973.

YESIL, E. E. et al. Urinary gamma-glutamyl transferase-to-creatinine ratio as an indicator of tubular function in Bence Jones Proteinuria. Renal Failure, v. 36, n. 3, p. 390-392, 2014.