GAMMA GT LIQUIFORM VET

Instructions for use

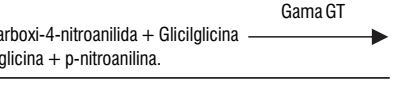
Ref.: **1058**

Purpose . System for quantitative determination of gamma-glutamyl transferase (Gamma GT) activity in serum, plasma (EDTA) and urine by photometry in kinetic mode.

Professional use only.

[For *in vitro* diagnostic use only.]

Principle . Gamma GT catalyses the transfer of the glutamyl group from L-y-glutamyl-3-carboxy-4-nitroanilide to glycylglycine, forming L-y-glutamylglycylglycine and p-nitroaniline, according to the following reaction:



The formed amount of released p-nitroaniline that presents high absorbance at 405 nm is directly proportional to the activity of Gamma GT in the sample.

System characteristics . Labtest developed the Gamma GT Liquiform VET system based on the principle of the modified Szasz method, which provides a modified procedure for the determination of Gamma GT. The performance of the method is substantially equivalent to the reference method proposed by the *International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)*[1,2] with analytical validation established by the *American Society for Veterinary Clinical Pathology (ASVCP).*[3]

The reaction components are properly distributed in two reagents to provide greater stability in the original liquid form and maintenance of optimal reaction conditions, allowing the direct use of the reagents (bi-reagent system) in automatic analyzers. Alternatively, a Working Reagent can be prepared that presents high stability (21 days between 4 and 8 ºC), allowing its use even in situations of low test demands. The system allows you to prepare the volume of Working Reagent required for a single measurement of GT Gamma enzymatic activity.

Gamma GT Liquiform VET has a linear response up to 700 U/L, equivalent to approximately 100 times the upper reference value in dogs and cats and 25 times the upper reference value in horses, significantly reducing the need for dilutions and repeat measurements on samples with high activities.

Measurements can be performed in continuous kinetic mode, both in automatic and semi-automatic analyzers. To this end, Labtest offers the Calibra VET line with Gamma GT activity traceable to the reference material ERM®-AD452/IFCC from the Institute for Reference Materials and Measurements and to the reference method of the IFCC.[2]

Methodology . Modified Szasz.

Reagents

1. R 1 **Reagent 1 - Store at 2 - 8 ºC.**
Contains glycylglycine 197 mmol/L and sodium azide x14.6 mmol/L and surfactant.

2. R 2 **Reagent 2 - Store at 2 - 8 ºC.**
Contains buffer s50 mmol/L L-gamma-glutamyl-3-carboxy-4-nitroanilide 21 mmol/L and sodium azide s14.6 mmol/L and stabilizers.

Unopened reagents, when stored under the indicated conditions, are stable until the expiration date printed on the label. Once opened, the reagents must be handled in accordance with good laboratory practices to avoid contamination of a chemical and microbial nature that could lead to reduced stability.

Precautions and warnings

Do not use the Working Reagent when its absorbance, measured against water at 405 nm is ≥ 1.5 or when it is turbid or shows signs of contamination.

In automatic analyzers, the reagents are subject to contamination with other reagents or with ambient air, which depend on the characteristics of the equipment and the working conditions. These contaminations can result in reduced stability and calibration changes.

The usual safety precautions should be used when handling the reagent.

The reagents contain sodium azide which is toxic. Do not ingest and, in case of contact with eyes, wash immediately with a large amount of water and seek medical assistance.

Azide can form highly explosive compounds with lead and copper pipes. Therefore, use large volumes of water to discard the reagent.

Material needed and not supplied

1. Pipettes to measure samples and reagents.
2. Stopwatch.
3. Photometer with thermostatic cuvette capable of accurately measuring absorbance at 400 and 420 nm for continuous kinetic method.
4. Refractometer for measuring urinary density to determine urinary Gamma GT.

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Preanalytical influences . Neonates of all species, except foals, may show a physiological increase in serum Gamma GT activity 24 hours after colostrum ingestion. In puppies, this increase in activity is 30 times the upper limit in healthy adults and lasts for 10 days.[4]

Treatment with anticonvulsants (phenobarbital and phenytoin) and glucocorticoids may increase serum Gamma Gt activity.[5]

Donkeys and mules have Gamma GT activity in the serum 2 - 3 times higher than that of horses.[5]

In female dogs, an increase in Gamma GT activity in the urine during pregnancy was verified.[6]

Sample

A Standard Operating Procedure (SOP) should be created to establish adequate procedures for sample collection, preparation and storage. We emphasize that the errors due to the sample can be much larger than the errors that occurred during the analytical procedure.

Use serum or plasma (EDTA). Canine serum has stable enzymatic activity for 7 days at 25 ºC and 15 days if kept between 2 - 8 ºC or minus 20 ºC[7]. Urine should preferably be kept between 2 - 8ºC for 7 days and should not be frozen. Bacteriuria or bacterial contamination during urine collection may lead to decreased urinary Gamma GT activity.

Biological samples should be considered as potentially infectious. Therefore, when handling them, the established regulation for biosafety must be followed.

To dispose of reagents and biological material, we suggest applying local, state or federal environmental protection regulations.

Interferences[12]

Anticoagulants containing citrate, fluoride or oxalate inhibit Gamma GT activity and heparin as an anticoagulant may increase the activity of the enzyme.

Bilirubin values up to 20 mg/dL and 1300 mg/dL of triglycerides do not produce significant interference in the detection of Gamma GT. Unlike what occurs in human samples, hemoglobin concentrations greater than 50 mg/dL cause a marked decrease in Gamma GT activity in canine, equine and feline samples.

To evaluate the approximate concentration of hemoglobin in a hemolyzed sample, proceed as follows:

Dilute 0.05mL of sample in 2.0mL of NaCl 150 mmol/L (0.85%) and measure absorbance at 405 or 415 nm, setting zero with deionized or distilled water.

Hemoglobin (mg/dL) ≅ $Absorbance_{405}$ x 601
Hemoglobin (mg/dL) ≅ $Absorbance_{415}$ x 467

Preparation of the working reagent . The set of a bottle of Reagent 1 and a bottle of Reagent 2 allows to prepare the Working Reagent. Transfer the contents of a Reagent 2 vial to a Reagent 1 vial and mix by inversion. Note the expiration date.

Stable 1 day at 15 - 25 ºC and 21 days at 2 - 8 ºC when there is no chemical or microbial contamination. Identify the Working Reagent bottle to avoid confusion with other Reagent 1 bottles. To preserve performance, the reagent should remain outside the storage temperature only as long as necessary to obtain the volume to be used. Avoid exposure to direct sunlight.

Optionally, a smaller volume of Working Reagent can be prepared using the proportion of four volumes of Reagent 1 and one volume of Reagent 2.

The Working Reagent contains 4.2 mmol/L L-gamma-glutamyl-3-carboxy-4-nitroanilide, 157.6 mmol/L glycylglycine, mmol/L sodium azide.

Procedure

Urinary centrifugation[8] . The determination of Gamma GT activity in urine must be preceded by centrifugation at 3500 rpm for 15 minutes, using the supernatant to measure urinary Gamma GT.

Continuous kinetic method . See items Linearity, Calibration and Observations 1, 2 and 3.

It is common practice to calculate enzyme activity results using a factor obtained under optimal reaction conditions that include: wavelength at 405 nm; cuvette thermostated at 37 ± 0.2 ºC with 1.0 cm thick solution; passband <2 nm and stray light <0.1.

As most of the time it is not possible to work under these conditions, good laboratory practices recommend performing the assay calibration using the enzyme calibrator indicated by the reagent manufacturer. Labtest indicates the Calibra VET line for calibration of the Gamma GT Liquiform system.

1. In a tube labeled "Test" or "Calibrator", pipette 1.0 mL of the Working Reagent.

2. Add 0.05 mL of sample or enzyme calibrator, homogenize and immediately transfer to the thermostated cuvette at 37 ± 0.2 ºC. Wait 1 minute.

3. Read the initial absorbance (A1) at 405 nm (400 - 420 nm), simultaneously starting the stopwatch. Repeat the reading after 2 minutes (A2).

To verify the linearity of the reaction, also take a reading with an interval of 1 minute and check if the difference in absorbance in each minute is constant.

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Calculations

ΔA Test or Calibrator $= (A_2 - A_1)/2$

$$\text{Factor} = \frac{\text{Calibrator Activity}}{\Delta A \text{ Calibrator}}$$

Gama GT (U/L) $= \Delta A$ Test x Factor

Example

Test
$A_1 = 0{,}872$ $A_2 = 0{,}944$

$$\Delta A \text{ Test} = \frac{0{,}944 - 0{,}872}{2} = 0{,}036$$

Calibrator
$A_1 = 0{,}852$ $A_2 = 0{,}904$

$$\Delta A \text{ Calibrator} = \frac{0{,}904 - 0{,}852}{2} = 0{,}026$$

Calibrator Activity (U/L): 67

$$\text{Factor} = \frac{67}{0{,}026} = 2577$$

Gamma GT Activity (U/L) of the sample $= 0.036 \times 2577 = 93$

When the optimal reaction conditions, mentioned above, are met, you can choose not to use the enzyme calibrator and apply the factor 2550. This factor was obtained under experimental conditions of the measurement system using a continuous chain of comparisons (traceability) with the IFCC reference method.[2]

Urinary Gamma GT . To correct the Gamma GT activity value according to the urinary flow, the urinary density of 1.025 is used as a correction factor through the following formula[9]:

$$\text{Corrected urinary GGT} = \frac{\text{Urinary GGT} \times 25}{\text{Last 2 digits of sample urine density}}$$

Example

Urinary Gamma GT = 30 U/L
Urinary density = 1.020

$$\text{Corrected urinary GGT} = \frac{30 \times 25}{20} = 37{,}5$$

Corrected urinary GGT = 37.5 U/L

Calibration

Manual calibrations . Continuous Kinetic Method: use Calibra VET calibrator. Gamma GT activity in Calibra VET is traceable to the ERM-AD452/IFCC reference material and the IFCC reference method.[2]

Automatic systems . Reagent blank: water or 150 mmol/L sodium chloride solution (0.85%); use calibrators from the Calibra VET line. Gamma GT activity in the Calibra VET line calibrators is traceable to the ERM-AD452/IFCC reference material and the IFCC reference method.[2]

Calibration interval

2-point calibration when changing batches;
2-point calibration when internal quality control indicates.

Linearity . The measurement result is linear up to 700 U/L. For higher values, dilute the sample with NaCl 150 mmol/L (0.85%), perform a new measurement and multiply the result obtained by the dilution factor. Dilute the sample in such a way that the value found is between 50 and 400 U/L. We suggest checking the methodological and photometric linearity, at least every six months, using samples with values up to 700 U/L.

Internal quality control . The laboratory must maintain an internal quality control program that clearly defines applicable regulations, objectives, procedures, criteria for quality specifications and tolerance limits, corrective actions and activity recording. Control materials should be used to evaluate inaccuracy and deviations from calibration. A state of control goal should be created and it is recommended that the specifications for the total error be based on what is established by the *American Social*, for *Veterinary Clinical Pathology (ASVCP)*.[3]

It is suggested to use the Qualitrol 1 VET product (Ref. 1014) by Labtest for internal quality control in clinical chemistry assays.

Methodological sensitivity . A protein sample not containing Gamma GT was used to calculate the limit of detection of the assay, finding a value equal to 2.48 U/L, equivalent to the mean of 20 assays plus two standard deviations. Using the minimum detectable absorbance as a parameter, the photometric sensitivity is 2.54 U/L, corresponding to an absorbance difference equal to 0.001.

Reference values . These values should be used as a guide only. It is recommended that each laboratory establish its own range of reference values in the served population.

Serum Gamma Gt[10]

Species	Gamma GT (U/L)
Canine	1.2 - 6.4
Feline	1.3 - 5.1
Equine	8 - 29

It is recommended that samples with Gamma GT enzymatic activity less than or equal to 2.48 U/L be reported as: “Gamma GT 2.48 U/L”. As healthy dogs and cats present the lower limit of the reference interval below the sensitivity of the test, it is common to obtain results with O U/L values or “no reading”, mainly in feline samples.[12]

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Urinary Gamma GT corrected for urine density[9,11]

Species	Gamma GT (U/L)
Canine	13 - 92
Feline	9 - 30

Conversion: Conventional Units (U/L) X 16.7 = SI Units (nkat/L)

Performance characteristics[12]

Recovery studies . In two samples with Gamma GT values equal to 295 and 485 U/L, amounts of the enzyme were added, obtaining the following results:

Activity	Initial (U/L)	295	485
	Added (U/L)	101	101
	Expected (U/L)	396	586
	Found (U/L)	391	585
	Recovery(%)	98.7	99.8

The estimated mean proportional systematic error equals 0.4 U/L for the 59 U/L decision level and 1.3 U/L for the 181 U/L decision level.

Methods comparison studies

Serum Gamma GT . The proposed method was compared with the IFCC[2] reference method, with the following results being obtained:

	Comparative Method	Labtest Method
Number of samples	80	80
Concentration range (U/L)	14,7 - 244,0	11,4 - 241,4
Average of estimates (U/L)	119,9	118,8
Regression equation	Labtest method = 1.015 x Comparative method = 2.97	
Correlation coefficient	0,999	

Using the regression equation, the estimated total systematic error (bias) equals -3.5% for a decision level equal to 59 U/L and -0.14% for a decision level equal to 181 U/L.

Urinary Gamma GT . The proposed method was compared with the similar method, and the following results were obtained:

	Comparative Method	Labtest Method
Number of samples	26	26
Concentration range (U/L)	6.51 - 119.0	6.16 - 93
Average of estimates (U/L)	43.4	36.91
Regression equation	Método Labtest = 0.8102 x Método Comparativo + 1.7887	
Correlation coefficient	0.9931	

Using the regression equation, the estimated total systematic error (bias) equals 15 % for a decision level of urinary Gamma GT equal to 30 U/L and 21 % for a decision level of urinary Gamma GT equal to 92 U/L.

Precision Studies . Precision studies were performed in the Labtest/Labmax 240® system, using samples with activity values equal to 59 and 181 U/L.

Repeatability - intra-assay inaccuracy

	N	Average	SD (U/L)	CV (%)
Sample 1	20	59	0,7	1,13
Sample 2	20	181	1,0	0,57

Reproducibility - total inaccuracy

	N	Average	SD (U/L)	CV (%)
Sample 1	20	59	1,5	2,58
Sample 2	20	181	3,2	1,80

Total error evaluation . The calculation of the total error was performed according to the established by the *American Society for Veterinary Clinical Pathology* (ASCVP)[3] through the following equation: Total error = 2 x Coefficient of variation (%) + Bias (%).

The estimated total error in the value of 59 U/L is equal to 8.8% and in the value of 181 U/L it is equal to 3.7%. The results indicate that the method meets the optimal specification for total error (20 %) based on the requirements established by ASVCP.[3]

Matrix dilution effects . Two samples with values equal to 710 and 603 U/L were used to evaluate the system response to matrix dilutions with 150 mmol/L NaCl (0.85%). Using dilution factors ranging from 2 to 16, an average recovery of 99.0% was found.

Clinical significance . Gamma GT is a cell membrane-associated enzyme and is present in high concentrations in biliary epithelial cells. When cholestatic hepatobiliary diseases occur, associated or not with biliary hyperplasia, the constituents of bile stimulate the synthesis and release of Gamma Gt.[13]

In horses, increased serum Gamma GT activity is primarily associated with cholangiohepatitis, cholelithiasis and hepatocellular necrosis (lesser elevation). The increase in Gamma GT has a magnitude greater than that of Alkaline Phosphatase, which is why it is an indicator with better diagnostic sensitivity to detect cholestasis in this species.[5,13]

In dogs, the increase in Gamma GT and Alkaline Phosphatase tends to occur concomitantly[13], however, Gamma GT is a more specific indicator of cholestasis[14]. Gamma GT values may be altered in dogs with liver diseases by treatment with steroids[13]. In cats, the increase in Gamma GT occurs before that of Alkaline Phosphatase, being a more sensitive indicator of liver disease (except in hepatic lipidosis, in which Gamma GT values may remain within the reference range).[5]

Gamma GT is also present in renal tubular cells and the presence of enzyme activity in the urine is an early indicator of acute kidney injury and a persistent marker of it. Its increase precedes changes in renal function tests[15]. There is no correlation between the increase in Gamma GT in serum and urine.

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Notes

1. Proper cleaning and drying of the material used are fundamental factors for the stability of the reagents and obtaining correct results.

2. The water used in the laboratory must be of adequate quality for each application. Thus, to prepare reagents and use in measurements, it must have a resistivity megaohm.cm or conductivity <1 microsiemens/cm and a silicate concentration <0.1 mg/L (type II water). For rinsing glassware, the water can be type III, with a resistivity k0.1 megaohms or a conductivity <10 microsiemens. For the final rinse, use type II water. When the deionizing column is saturated, alkaline water is produced with the release of various ions, silicates and substances with great oxidation or reduction power that deteriorate the reagents in a few days or even hours, changing the results unpredictably. Thus, it is essential to establish a water quality control program.

3. As with any measurement of enzymatic activity, the rigorous observation of the time and temperature of incubation is of great importance for the quality of the results obtained.

References

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Presentation

Product	Reference	Content	
GAMMA GT LIQUIFORM VET	1058-2/30	Reagent 1	2 X 24 mL
		Reagent 2	2 X 6 mL

Applications for automatic and semi-automatic systems are available.

The number of tests in automatic applications depends on the programming parameters.

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Consumer information

[Guarantee Terms and Conditions]

Labtest Diagnóstica guarantees the product performance, within the specifications, until the expiration date indicated on the labels, provided that the care for use and storage indicated on the labels and in these instructions are correctly followed.

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro . Symbols used with ivd devices

Conteúdo suficiente para < n > testes Contenido suficiente para < n > tests Contains sufficient for < n > tests	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	**Controle** Control Control	**Tóxico** Tóxico Poison
Data limite de utilização (aaaa-mm-dd ou mm/aaaa) Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	**Número do catálogo** Número de catálogo Catalog Number	**Controle negativo** Control negativo Negative control	**Reagente** Reactivo Reagent
Material Calibrador Material Calibrador Calibrator Material	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes	**Controle positivo** Control positivo Positive control	**Fabricado por** Elaborado por Manufactured by
Material Calibrador Material Calibrador Calibrator Material	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device	**Controle** Control Control	**Número do lote** Denominación de lote Batch code
Limite de temperatura (conservar a) Temperatura limite (conservar a) Temperature limitation (store at)	**Liofilizado** Liofilizado Lyophilized	**Risco biológico** Riesgo biológico Biological risk	**Período após abertura** Periodo post-abertura Period after-opening
Representante Autorizado na Comunidade Europeia Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	**Corrosivo** Corrosivo Corrosive	**Marca CE** Marcado CE CE Mark	**Uso veterinário** Uso veterinario Veterinary use
Instalar até Instalar hasta Install before	**Fabricado em** Elaborado en Manufactured on	**Produto de uso único** Producto de un solo uso Single use product	Ref.: 280322