FRUCTOSAMINE VET
Instructions for use

Ref.: **1019**

Intended purpose . Fructosamine determination system by fixed-time kinetic method in serum samples.

Professional use only.

[Only for *in vitro* diagnostic use]

Principle . Glucose present in serum binds to plasma proteins forming a Shiff base, which after a molecular rearrangement, becomes a stable ketoamine generically called fructosamine1. The quantification of fructosamine reflects the glycemic level of the animals in the last 7 to 15 days, without being influenced by hyperglycemia caused by food and stress at the time of collection, recurrent situations in veterinary medicine.[2]

At alkaline pH, fructosamine is converted to the enol form, which reduces nitrotetrazolic blue to a "purple formazen". The measurement of the difference in absorbance after incubation is proportional to the concentration of fructosamine in the sample. The system calibration is carried out with a bovine matrix calibrator, calibrated with glycated polylysine. Results are expressed in micromolesitometers (umol/L).

System characteristics . The determination of fructosamine, formed by the binding of glucose to plasmatic proteins, is based on its reducing capacity in an alkaline medium. Uric acid in serum is a reducing agent that can interfere with the assay. Fructosamine VET (Ref. 1019) has the enzyme Uricase in Reagent 1, minimizing uric acid interference in the assay.

Calibration with a bovine matrix calibrator means that small variations in test conditions do not produce significant interference3. The benefits of this system are more consistent results, with greater reproducibility and accuracy.

The system uses a Working Reagent stable for 30 days, when stored between 2 - 8 ºC, also allowing to prepare the volume of Working Reagent necessary for a single measurement of fructosamine concentration. Reagents must not remain open for a prolonged period, as exposure to atmospheric air may lead to a decrease in their stability.

The method is easily applicable to automatic and semiautomatic analyzers capable of measuring, in kinetic mode, absorbance differences at 530nm.

Methodology . NBT reduction

Reagents

1. R 1 - Reagent 1 -Store between 2 - 8 ºC.
Contains buffer 83 mmol/L pH 7.3; nitrotetrazolium blue (NBT) 967 p.mol/L; uricase 5000 U/L; sodium azide 14.6 mmol/L; surfactants and stabilizers.

2. R 2 - Reagent 2 -Store between 2 - 8 ºC.
After handling, store tightly sealed. Contains 625 mmol/L buffer pH 10.4 and 14.6 mmol/L sodium azide.

3. CAL - Calibrator - Concentration on the vial label. Store between 2 - 8ºC.
Lyophilized reagent. Contains glycated bovine albumin; buffer 50 mmol/L, pH 7.4 and sodium azide 14.6 mmol/L.

Unopened reagents, when stored under the indicated conditions, are stable until the expiration date printed on the label. While handling, reagents are subject to chemical and microbial contamination that can cause reduced stability.

Precautions and warnings

Keep open reagents only for the time necessary to perform the test. Exposure to atmospheric air reduces the stability of the reagents, unpredictably compromising the result.

Control of temperature and incubation time during the test must be rigorous. A difference of 1 ºC in temperature introduces an error of 5%, while a difference of 1 minute during the measurement of A produces an error of 20%.

Usual safety precautions should be applied when handling reagents, which should not be pipetted by mouth.

The reagents contain sodium azide which is toxic. Special care must be taken to avoid ingestion and, in case of eye contact, wash your eyes immediately with abundant 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 reagents.

Material needed and not supplied

1. Water bath maintained at constant temperature (37 ºC).
2. Photometer capable of accurately measuring absorbance between 510 and 550 nm.
3. Pipettes to measure samples and reagents.
4. Stopwatch.

Pre-analytical influences . The use of Levodopa may cause falsely increased results of Fructosamine.[4]

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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 (Heparin or EDTA). Dog sera are stable for 5 days at 4 ºC or 25 ºC and for 28 days when kept at -20 ºC.[5]

Since no known test can guarantee that blood samples do not transmit infections, they should all 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.

Interference[6]

Hemoglobin concentrations greater than 100 mg/dL in dog and horse sera produce a significant increase in fructosamine quantification. In felines, this increase occurs in samples with hemoglobin greater than 50 mg/dL.

To evaluate the approximate concentration of hemoglobin in a hemolyzed sample, proceed as follows: dilute 0.05 mL of the sample in 2.0 mL of NaCl 150 mmol/L (0.85%) and measure the absorbance at 405 nm or 415 nm by adjusting to zero with deionized or distilled water.

$$\text{Hemoglobin (mg/dL)} \cong \text{Absorbance}_{405} \times 601$$
$$\text{Hemoglobin (mg/dL)} \cong \text{Absorbance}_{415} \times 467$$

Bilirubin concentrations up to 5 mg/dL and glucose up to 1000 mg/dL do not interfere significantly. Turbid samples, mainly due to lipemia, should not be used, as they could lead to falsely reduced results.

Reagent preparation

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. Identify the Working Reagent vial and note the expiration date. The Working reagent can be used for 30 days if stored between 2 - 8 ºC, when there is no chemical or microbial contamination (see Calibration item).

The working reagent is an alkaline solution (pH = 10.3) and as such is unstable when exposed to atmospheric air. Therefore, this reagent can have its performance compromised, in an unpredictable way, if kept open, either in the automatic analyzer or on the bench. To preserve performance, the working reagent should remain open only as long as necessary to obtain the volume to be used. Store tightly sealed.

Optionally, a smaller volume of Working Reagent can be prepared using the proportion of 3 (three) volumes of Reagent 1 and 2 (two) volumes of Reagent 2.

Example . to prepare 10 mL of Working Reagent, mix 6 mL of Reagent 1 and 4 mL of Reagent 2.

The Working Reagent contains 50 mmol/L phosphate buffer, 250 mmol/L carbonate buffer, 580 Imol/L nitrotetrazolium blue, k3000 U/L uricase, detergents and stabilizers at pH 10.3.

Calibrator . Reconstitute the contents of the calibrator bottle (Reagent 3) with 2.0 mL of distilled or deionized water and leave it to rest for 30 minutes. Homogenize by inversion. Stable for 60 days between 2 - 8 ºC and 6 months at minus 10 ºC. Homogenize before use.

Procedure

This procedure applies to semi-automatic analyzers that use only a flow cell. Applications for automatic and semi-automatic systems are available.

See notes 1, 2 and 3.

Label 2 test tubes as "Test" and "Calibrator" and proceed as follows:

	Test	Calibrator
Working Reagent	1.0 mL	1.0 mL

Incubate at 37ºC for 2 minutes

Sample	0.050 mL	---------
Calibrator	---------	0.050 mL

Mix well and incubate at 37 ºC. After exactly 10 minutes (timed) determine the absorbances (A_1) of the test and the 530 nm calibrator (510 - 550), adjusting the zero with distilled or deionized water. Continue the incubation at 37 ºC for exactly 5 minutes (timed) and determine the absorbances (A_2) of the test and the calibrator at 530 nm (510 - 550), adjusting the zero with distilled water).

The suggested measurement procedure is suitable for photometers whose minimum solution volume for reading is equal to or less than 1.0 mL. A check should be made on the need to adjust the volume for the photometer used. Sample and reagent volumes can be modified proportionally without impairing the test performance and the calculation procedure remains unchanged. In case of reduction of volumes, it is essential to observe the minimum volume required for photometric reading. Sample volumes less than 0.01 mL are critical in manual applications and should be used with caution because they increase measurement inaccuracy.

Calculations . See item Linearity

Calculate absorbance differences for the Test and Calibrator: $\Delta A = A_2 - A_1$

Fructosamine (µmol/L) = (ΔA Test / ΔA Calibrator) x Calibrator concentration

Example

Test Absorbance

$A_1 = 0.211$ $A_2 = 0.252$ ΔA Test = 0.041

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Calibrator absorbance
$A_1 = 0.214$ $A_2 = 0.275$ ΔA Test = 0.061

Calibrator concentration in use = 359 μmol/L

Fructosamine (μmol/L) = (0.041/0.061) x359 = 241 μmol/L

Due to the high reproducibility that can be obtained with the methodology, the factor method can be used.

Calibration factor = Calibrator concentration/ AA Calibrator

Fructosamine (μmol/L) = AA Test x Factor

Calibration factor = 359/0.061 = 5885
Fructosamine (μmol/L) = 0.041 x5885 = 241 μmol/L.

Calibration . Calibrator concentration is traceable to a protein standard calibrated with $^{14}C^6$-labelled glucose-glycated polylysine sample.

Manual calibrations
2 point Calibration
Calibrator included (Ref. 1019.3)

Automatic systems
2-point calibration;
Reagent blank: deionized water or 150 mmol/L NaCl;
Calibrator included (Ref. 1019.3)

Calibration interval
The system must be calibrated weekly and in the following situations:
When internal quality control indicates;
When using a new batch of reagents;
When using a new working reagent.

Linearity

The measurement result is linear between 20 thymol/L and 800 μmol/L. For higher values, dilute the sample with NaCl 150 mmom/L (0.85%), perform a new measurement and multiply the result obtained by the dilution factor.

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.

It is suggested that the specifications for the coefficient of variation and the total error be based on that established by the *American Society for Veterinary Clinical Pathology* (ASVCP).[7]

Reference interval[4,8] . These values should be used as a guide only. It is recommended that each laboratory establish, in the population of animals treated, its own range of reference values.

Fructosamine (μmol/L)

Canines	274 - 382
Felines	174 - 294
Equines	235 - 332

Performance characteristics[6]

Accuracy . In two samples with fructosamine concentrations equal to 175 and 253 μmol/L, a known amount of analyte was added, obtaining an average recovery of 102%. The estimated mean proportional systematic error is equal to 5.1 μmol/L for the decision level of 256 μmol/L (feline) and 7.8 μmol/L for the decision level of 388 μmol/L (canine and equine).

Specificity . The proposed method was compared with a similar method using 40 samples with values between 33 and 775 μmol/L. The comparison resulted in the regression equation y= 1.029 x - 1.346 and a correlation coefficient (r) equal to 0.998. An extremely positive correlation between the two methods is evident, with a systematic error of 2.3% for the decision level 256 μmol/L (feline) and 2.6% for the level of 388 μmol/L (canine and equines).

Repeatability - intra-assay inaccuracy

	N	Average	SD	CV (%)
Sample 1	20	256	4.09	1.60
Sample 2	20	388	3.24	0.84

Reproducibility - total inaccuracy

	N	Average	SD	CV (%)
Sample 1	20	256	4.73	1.85
Sample 2	20	388	8.21	2.12

Total error evaluation . The calculation of the total error was performed according to the established by the *American Society for Veterinary Clinical Pathology* (ASCVP)[7] through the following equation:

Total error = 2 x Coefficient of variation (%) + Bias (%).

The estimated total error in the value of 256 μmol/L is equal to 3.72% and in the value of 380 μmol/L it is equal to 2.17%. The results indicate that the method meets the desirable specification by the ASVCP for the desirable total error (<9.6%).[7]

Methodological sensitivity . A sample of NaCl 150 mmol/L (0.85%) was used to calculate the detection limit of the assay, finding a value equal to 5.0 μmoUL, equivalent to the mean of 20 assays plus two standard deviations. It was found that the limit of photometric detection is 3.2 μmon corresponding to an absorbance equal to 0.001.

Matrix dilution effects . A sample with a value equal to 658 μm VL was used to evaluate the system response to matrix dilutions with 150 mmol/L NaCl (0.85%). Using various dilution factors, an average recovery of 101% was found.

Clinical significance . Fructosamine is the generic name given to

glycated proteins resulting from the binding of blood glucose to plasma proteins. This glycation is dependent on the serum concentration of glucose and proteins, and seems to be linked especially to albumin in dogs and to globulins in cats[9]. Fructosamine dosage reflects plasma glucose levels in one or two weeks prior to collection. The measurement of fructosamine is considered the gold standard test for the assessment of blood glucose in cats because it is not influenced by hyperglycemia resulting from stress (acute or chronic), which is very common in cats.[11]

The elevation of fructosamine levels occurs in Diabetes mellitus and its quantification is essential to monitor the efficiency of insulin therapy[4]. In dogs and cats, a fructosamine concentration between 350 - 400 μmoVL indicates an excellent response of the animal to insulin therapy, while fructosamine levels above 500 llmol/L indicate an inefficient glycemic control.[12]

The reduction of fructosamine concentration may occur in cases of persistent hypoglycemia caused by pancreatic neoplasia (insulinoma) and in cats with hyperthyroidism[4]. Additionally, fructosamine levels may be reduced in severe hypoproteinemia in cats and hypoproteinemia associated with severe hypoalbuminemia in dogs.[4'9]

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 clinical laboratory aims to provide accurate and precise results. The use of water of inadequate quality is a potential cause of analytical errors. The deionized or distilled water used in the laboratory must be suitable for each application. Thus, to prepare reagents, to use in measurements, and for use in final rinsing of glassware, it must have resistivity > 1 megaohm.cm or conductivity < 1 microsiemens/cm and silicate concentration <0.1 mg/L. 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.

References

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Presentation

Product	Reference	Contents	
Fructosamine Vet	1019-3/15	R 1	3 X 9 mL
		R 2	3 X 6 mL
		CAL	1 X 2 mL

For information about other commercial presentation visit the website www.labtest.com.br or contact Customer Assistance Service.

Applications for automatic and semi-automatic systems are available.

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

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		**Risco biológico** Riesgo biológico Biological risk
	**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)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Período post-abertura Period after-opening		**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		

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