URIC ACID Liquiform

Insert

Ref.: 140

Purpose . Enzymatic system for determination of uric acid by endpoint reaction in blood, urine and fluids (synovial and amniotic fluids).

Professional Use.

[For in vitro diagnostic use]

Principle . Uric acid is determined according to the following reactions:

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + \text{DHBS} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Antipirilquinonimine} + 4\,H_2O$$

Uric acid is oxidized by uricase to allantoin and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with 4-aminoantipyrine and DHBS, forming the chromogen antipirilquinonimine. The intensity of the color generated is directly proportional to the uric acid concentration in the sample.

System features . The determination of uric acid using the Trinder reaction is characterized as being a direct method of easy application on automated systems, which has the uricase specificity. Many products employ phenol as coupling reagent. However, the low sensitivity of phenol and the optimum pH incompatibility between the animal uricase and peroxidase result in serious obstacles to the reliability of the method.

The Labtest with the Uric Acid Liquiform system overcomes these difficulties by replacing phenol by 3,5-dichloro-2-hydroxybenzene sulfonate acid (DHBS), which is 4 times more sensitive, allowing an appropriate relationship between samples and reagents, enabling to obtain an optimal sensitivity with respect to low concentrations of analyte.

The reagent is provided in liquid form and is distributed in two reagents, enabling its use in automated systems and facilitating the elimination of interferences.

One-reagent methodology can be applied by using a Working Reagent stable until the expiration date of reagents composing it, when maintained between 2 and 8ºC. The Working Reagent has adequate performance even in situations of low demand. The system also enables the preparation of the Working Reagent volume required for measuring the concentration of uric acid.

The linearity of the method is of 20 mg/dL, which decreases the need to perform dilutions in a significant number of samples.

The reagent was developed with a special set of surfactants that promote clarification of turbidity caused by lipids, which significantly reduces the interference caused by hyperlipemia on the determination of the concentration of uric acid.

The proposed method is easily applicable to most automated and semi-automatic analyzers capable of measuring an endpoint reaction between 490 and 540 nm.

Methodology . Enzymatic - Trinder.

Reagents

1. R 1 - Reagent 1 - Store at 2-8ºC

Contain 155 mM buffer; 4-aminoantipyrine ≥0.1 mM; peroxidase ≥1,000 U/L; 0.02% sodium azide and surfactants.

2. R 2 - Reagent 2 - Store at 2-8ºC

Contain 155 mM buffer; DHBS ≥2.5 mM; uricase ≥300 U/L; 0.02% sodium azide and surfactants.

3. CAL - Standard - Store at 2-8ºC

Contain 6.0 mg/dL uric acid. Store tightly sealed to prevent evaporation.

Unopened reagent, when stored under the specified conditions, is stable until the expiration date printed on label. During handling, reagents are subject to contamination of chemical and microbial nature which may result in reduced stability.

Preparation of working reagent . The set containing a bottle of Reagent 1 and a bottle of Reagent 2 allows preparing the Working Reagent. Transfer the contents of one vial of Reagent 2 to a vial of Reagent 1 and mix gently. Identify the bottle containing the Working Reagent and note the expiration date.

The working reagent is stable for 14 days since it is kept between 2 and 8 ºC in a closed container and when there is no chemical or microbial contamination. The slightly pink color development in Working Reagent is normal and does not affect its performance.

Optionally, you can prepare a lower volume of Working Reagent by using the ratio of 4 volumes of Reagent 1 and 1 volume of Reagent 2. To prepare the volume of reagent required to perform a test, mix 0.8 mL of Reagent 1 and 0.2 mL of Reagent 2.

To maintain the performance, the reagent must remain outside the refrigerator only for the time required to obtain the volume to be used. Avoid exposure to direct sunlight.

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Precautions and special care

Do not use the reagent if its absorbance measured at 505 nm against water is equal to or greater than 0.300, or when it is cloudy and showing signs of contamination.

Usual safety care should be applied when handling the reagent. The reagents contain sodium azide which is toxic. Do not ingest, and in case of contact with eyes, wash them immediately with plenty of water and seek medical advice. Azide can form highly explosive compounds when in contact with lead or copper pipes. Therefore, use large amounts of water for disposing the reagents.

Care with reaction time, temperature and pipetting work is extremely important to obtain correct results.

Reagents must be handled in accordance with good laboratory practices, which include avoiding the ingestion and contact with skin, eyes and mucous membranes.

Material required and not provided

1. Water bath or incubator maintained at constant temperature (37ºC).

2. Photometer able to accurately measure the absorbance between 490 and 540 nm.

3. Pipettes for measuring samples and reagents.

4. Chronometer.

Pre-analytical influences . Uric acid is increased within the 24 hours following the ingestion of alcohol.

Serum concentrations of uric acid have wide day-to-day and seasonal variations in the same individual. The uric acid increases under stress, states of starvation and increased body weight.

Ascorbic acid (vitamin C), a reducing substance, consumes hydrogen peroxide, which leads to results falsely decreased. The patient should be instructed to not eat foods or use products containing ascorbic acid up to 48 hours before the examination[12].

Sample

Use serum, plasma (EDTA, heparin), urine and fluids (amniotic and synovial). The analyte is stable for 3 days at 2 to 8ºC and 6 months at 10ºC negative.

It should be elaborated a Standard Operating Procedure (SOP) for collection, preparation and storage of the sample. We emphasize that the errors arising from sample can be much larger than errors occurring during the analytical procedure.

Since no known test can ensure that blood samples do not transmit infections, they all must be considered potentially infectious. Therefore, when handling them, you must follow the rules established for biosafety.

For disposal of reagents and biological material we suggest to apply the local, state or federal environmental protection standards.

Interference

The use of plasma containing fluoride leads to obtaining results falsely decreased. Fluoride acts as an inhibitor of uricase.

The two-reagent methodology regards the following values for interferences:

Bilirubin up to 5 mg/dL, hemoglobin up to 200 mg/dL, and triglyceride samples up to 1200 mg/dL do not produce significant interference.

The one-reagent methodology considers the following values for interferences:
Bilirubin up to 5 mg/dL, hemoglobin up to 50 mg/dL, and triglycerides samples up to 1200 mg/dL do not produce significant interference.

To evaluate the approximate concentration of hemoglobin in a hemolyzed sample, you can proceed as follows: Dilute 0.04 mL of sample in 2.0 mL of 150 mmol/L NaCl (0.85%) and measure the absorbance between 405 and 415 nm, hitting the zero with distilled or deionized water.

Procedure

Samples: Serum, plasma (EDTA, heparin), urine and fluids (amniotic and synovial).

Urine . Homogenize the urine, separate 10 mL, adjust pH between 7.0 and 9.0 with 5% NaOH and heat for 10 minutes at 56ºC to dissolve the uric acid and urate crystals. Dilute the urine at 1:10 (0.1 mL of urine + 0.9 mL of distilled water). Multiply the result obtained by 10.

Procedures for performing the test

Take 3 test tubes and proceed as follows:

	Blank	Test	Standard
Sample	----	0.02 mL	----
Standard (Nº 3)	----	----	0.02 mL
Working Reagent	1.0 mL	1.0 mL	1.0 mL

Mix and incubate in a water bath at 37ºC for 5 minutes. The water level in the water bath should be higher than the level of reagent in test tubes. Determine the test and standard absorbance at 505 nm or green filter (490 - 540 nm), hitting the zero with the blank. The color is stable for 30 min.

The suggested procedure for measuring is suitable for photometers with minimum volume of solution for reading equal to or less than 1.0 mL. It should be checked the need for adjusting the volume for the photometer used. The sample and reagent volumes can be modified proportionately without affecting the performance of the test and the calculation procedure remains unchanged. In case of reduced volumes, it is essential to observe the minimum volume required for photometric reading. Sample volumes smaller than 0.01 mL are critical in manual applications and should be used with caution because they increase the inaccuracy of the measurement.

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Calculations

$$\text{Uric Acid (mg/dL)} = \frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times 6$$

Example

Test Absorbance = 0.214
Standard Absorbance = 0.163

$$\text{Uric Acid (mg/dL)} = \frac{0.214}{0.163} \times 6 = 7.9$$

The result can also be obtained by using the calibration factor.

$$\text{Calibration Factor} = \frac{6}{\text{Standard Absorbance}}$$

Example

$$\text{Calibration Factor} = \frac{6}{0.163} = 36.8$$

$$\text{Uric Acid (mg/dL)} = 0.214 \times 36.8 = 7.9$$

$$\text{Urine (mg/24 hrs)} = \frac{\text{mg/dL x urine volume (mL)}}{100}$$

Calibration

System Traceability
The standard is traceable to Standard Reference Material (SRM) 913 of the National Institute of Standards and Technology (NIST).

Manual Calibrations
Obtain the calibration factor when using a new batch of reagents or when the internal quality control indicates.

Automated systems
Reagent blank: deionized water or 150 mmol/L (0.85%) sodium chloride solution;
Calibrator: use a protein calibrator. The concentration of uric acid in the calibrator Calibra H - Labtest is traceable to NIST SRM 913.

Calibration Range
When the internal quality control indicates;
When using a new reagent batch;
When using a new reagent bottle from the same batch when a new calibration has been performed during the use of the preceding bottle.

Measurement/reportable range

The measurement result is linear up to 20 mg/dL. When obtained a value equal to or greater than 20 mg/dL, dilute the sample with 150 mmol/L NaCl (0.85%), repeat the measurement and multiply the result by the dilution factor.

Urine. Dilute the sample (pH between 7.0 and 9.0 and heated for 10 minutes at 56ºC) to 1:20 or 1:40 with distilled or deionized water and repeat the measurement. Multiply the result by 20 (twenty) or 40 (forty), as previously used dilution.

Internal quality control . The laboratory should maintain a program of internal quality control, clearly defining regulations, objectives, procedures, criteria for quality specifications and tolerance limits, corrective actions and activities recording. Control materials should be used to evaluate inaccuracy and calibration deviations.

It is suggested that the specifications for the coefficient of variation and total error are based on the components of biological variation (BV)[9,10].

It is suggested to use stabilized preparations of Qualitrol H line - Labtest for internal quality control in clinical chemistry assays.

Reference range . The ranges should be used only as a guide. It is recommended that each laboratory establish its own reference ranges in the patient population.

Serum (mg/dL)

Children[11]	Men	1.5 - 6.0
	Women	0.5 - 5.0
Adults	Men	2.5 - 7.0
	Women	1.5 - 6.0

Urine . 250-750 mg/24 hours

Conversion . conventional units (mg/dL) x 59.5 = IS units (mmol/L)

Performance features[13]

Recovery study . In two samples with uric acid concentration equal to 5.9 and 6.3 mg/dL were added different amounts of analyte, obtaining recoveries between 99.6 and 100.2%.

The average total systematic error obtained for a sample with mean uric acid value of 8.0 mg/dL was equal to 0.01 mg/dL or 0.15%. The total systematic error obtained is smaller than the total systematic error of the desirable specification based on components of the Biological Variation, which is $\leq \pm 4.9\%$.

Study comparing methods . The proposed method was compared with another product of similar methodology, and the following results were obtained:

	Comparative Method	Uric Acid Liquiform
Number of samples	40	40
Concentration range (mg/dL)	1.92 - 15.79	1.99 - 16.27
Regression equation	Labtest Method (mg/dL) = 1.0322 x Comparative - 0.0844	
Correlation coefficient	0.9995	

The total systematic error observed in decision levels 2.0, 8.0 and 10.7 mg/dL was equal to 0.02, 0.17 and 0.26 or 1.00, 2.17 and 2.43%, respectively.

The total systematic error obtained is smaller than the total systematic error of the desirable specification based on components of the Biological Variation, which is ≤±4.9%.

Accuracy studies . Accuracy studies were conducted using 40 samples with mean concentrations equal to 2.1, 8.6 and 11.7 mg/dL.

Imprecision - Within run

	N	Mean	SD	%CV
Sample 1	40	2.3	0.02	0.97
Sample 2	40	9.5	0.07	0.85
Sample 3	40	11.1	0.09	0.82

Imprecision - Run-to-run

	N	Mean	SD	%CV
Sample 1	40	2.3	0.03	1.48
Sample 2	40	9.5	0.09	1.14
Sample 3	40	11.1	0.09	1.14

The total error (random error + systematic error) estimated in concentrations equal to 2.0, 8.0 and 10.7 mg/dL is equal to 3.45%, 4.04% and 4.32%, respectively.

The results indicate that the method meets the specification desirable for the total error (≤ ± 12.4%) based on desirable components of the Biological Variation.

Methodological sensitivity . A sample containing no uric acid was used to calculate the detection limit of the assay, having found a value of 0.02 mg/dL, which is equivalent to the mean of 10 assays plus three standard deviations.

Matrix dilution effects . Two samples with values equal to 24.9 and 23.2 mg/dL 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, it was found mean recoveries of 101%. The total systematic error obtained is smaller than the total systematic error of the desirable specification based on components of the Biological Variation, which is ≤±4.9%.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The water in the laboratory to prepare reagents and use in the measurements must have resistivity ≥1 megaohm.cm or conductivity ≤1 microsiems/cm and silicates concentration must be <0.1mg/L (Type II reagent water). The water for washing must be Type III, having resistivity ≥0.1 megaohms or conductivity ≤10 microsiemens. For the final washing, use Type II reagent water.

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13. Labtest: Data on file.

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Presentation

Product	Reference	Contents	
Uric Acid Liquiform	140-1/100	R 1	1 x 80 mL
		R 2	1 x 20 mL
		CAL	1 x 5 mL
	140-1/250	R 1	1 x 200 mL
		R 2	1 x 50 mL
		CAL	1 x 5 mL
Uric Acid Liquiform Labmax 560/400	140-4/64	R 1	4 x 51 mL
		R 2	4 x 13 mL
		CAL	1 x 5 mL

The number of tests **in automated systems depends of the programmed parameters.**

Application procedures are available for various automated systems.

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

CE

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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		

Ref.: 140214

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