



بسم الله الرحمن الرحيم



**Sudan University of Science and Technology**

**Collage of Medical Laboratory Science**

**Clinical Chemistry Department**

**Comparison between Two Different Methods (Caraway Chemical and Uricase  
Enzymatic) for Estimation of Uric Acid in Plasma**

مقارنه بين طريقتين مختلفتين (كاراوي الكيميائية واليوريكيز الانزيميه)  
لتقدير مستوى حامض البوليك في بلازما الدم

**A dissertation submitted in partial fulfillment for B.Sc (Honor) degree in  
medical laboratory science (Clinical Chemistry)**

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# الآية

قال تعالى:

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

"فَفَهَّمْنَاهَا سُلَيْمَانَ وَكَلَّمْنَا دَاوُدَ إِذْ أَنشَأَ لَدُنَّا دَاوُدَ رُجُلًا سَابِقًا إِلَىٰ مَا يَسْعَىٰ بِآيَاتِنَا وَجَعَلْنَا دَاوُدَ وَاللُّؤْلُقَ إِذْ يَخْتَصِمَانِ

يُسَبِّحُنَ وَالطَّيْرَ وَكُنَّا فَاعِلِينَ"

صدق الله العظيم

سورة الانبياء

الاية (79)

## *Dedication*

*To.....*

*Our family... father, mother, sisters, and brother*

*To.....*

*Our Friends and colleagues for sharing life with us*

*To.....*

*Our teachers who help and support us*

*To.....*

*People who participated fully and helped us a lot to achieve this work,*

*Special dedication to our supervisor Ustaz: Bader Aldeen Musa Ahmed Yousif*

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## Abstract

This study conducted during the period of ( February 2014 – August 2014) and aimed to compare between the old caraway chemical method and enzymatic method which are commonly used available uricase enzymatic method which are commonly used for measurement of uric acid in practice in sudan. This study include 50 samples collected from 50 volunteers who attended randomly. All samples collected in heparin containers and separated immediately.

Venous blood samples were taken from the study population and uric acid was measured by chemical and enzymatic methods.

All results were analyzed using SPSS program.

These study showed that there was statistically significant increase in the results of uric acid obtained by using uricase enzymatic method (mean=3.9mg/dl) than the results obtained by using caraway chemical method (mean=3.3mg/dl) with (P.value=0.027)

This study also reveal that there was statistically significant increases in uric acid levels (mean=4.4mg/dL) in males (n=24) , when compared with that of (n=26) females (mean=3.3mg/dL) with (P.value=0.001) by uricase enzymatic method and (mean=3.8 mg/dl) in male (n=24) when compared with that of (n=26) females (mean=2.9 mg/dl) with (P.value = 0.005) by caraway chemical method.

The uricase enzymatic and caraway methods can be recommended to use in sudan for uric acid measurement considering the following factors:Availability, cost, time consumed, which one easy to perform and reference value of each one

## ملخص الدراسة

هذه الدراسة اقيمت أثناء الفتره من شهر فبراير الى اغسطس 2014 وتهدف للمقارنه بين الطريقتين الكيميائيه والانزيميه الحديثه المتوفره في الشركات وهي الاكثر استخداما لقياس حامض اليوريك في السودان. شملت هذه الدراسه 50 عينه جمعت من 50 متبرع وجمعت عشوائيا خلال هذه الفتره. كل هذه العينات جمعت في حافظات تحتوي على مواد مانعه للتجلط وتم فصلها مباشره بلازما الدم استهلكت لقياس حامض اليوريك مرتين بالطريقه الكيميائيه والانزيميه، كل النتائج حلت باستخدام برنامج نظام الحزم الاحصائيه للعلوم الاجتماعيه. أظهرت هذه الدراسه انه يوجد زياده احصائيه معنويه في نتائج حمض اليوريك المتحصله باستخدام الطريقه الانزيميه الحديثه (اليوريكيز) (الوسط الحسابي=3.9) عند مقارنتها بالطريقه الكيميائيه القديمه (كاراوي) (الوسط الحسابي=3.3) مع قيمه احتماليه=0.027) هذه الدراسه اوضحت ان هناك زياده احصائيه معنويه في مستوى حامض اليوريك(الوسط الحسابي=4.4) ل24 عينه من الرجال عند مقارنتها ب 26 عينه من النساء(الوسط الحسابي=3.3) مع قيمه احتماليه=0.001 بالطريقه الانزيميه وايضا هناك زياده احصائيه معنويه في مستوى حامض اليوريك (الوسط الحسابي=3.8) ل24 عينه من الرجال عند مقارنتها ب 26 عينه من النساء (الوسط الحسابي=2.9) مع قيمه احتماليه=0.005 بالطريقه الكيميائيه. يمكن استخدام الطريقه الانزيميه(اليوريكيز)والكيميائيه(كاراوي)لتقدير حمض اليوريك في السودان مع الاخذ بعين الاعتبار العوامل التاليه:التكلفه،الزمن،ايهما سهله الاستخدام والمدى الطبيعي لكل منهما.

## Contents

| NO                 | Subjects                                 | Pages |
|--------------------|--|-------|
|                    | الإيه                                    | I     |
|                    | Dedication                               | II    |
|                    | Acknowledgements                         | III   |
|                    | Abstract (English)                       | IV    |
|                    | Abstract (Arabic)                        | V     |
|                    | Contents                                 | VI    |
|                    | List of tables                           | IX    |
|                    | List of figures                          | X     |
| <b>CHAPTER ONE</b> |  |       |
| 1.                 | Introduction and Literature Review       | 1     |
| 1.1.               | Uric Acid                                | 1     |
| 1.1.1.             | Definition                               | 1     |
| 1.1.2.             | Biochemistry and Physiology of Uric acid | 1     |
| 1.1.3.             | Disorders of abnormal uric acid          | 2     |
| 1.1.3.1.           | Genetics                                 | 2     |
| 1.1.3.2.           | Hyperuricemia                            | 2     |
| 1.1.3.3.           | Hypouricemia                             | 5     |

|                    |                         |    |
|--------------------|-------------------------|----|
| 1.1.3.3.1.         | Causes                  | 6  |
| 1.1.4.             | Estimation of uric acid | 7  |
| 1.1.5.             | Objectives              | 8  |
| 1.1.5.1.           | General objective       | 8  |
| 1.1.5.2.           | Specific objectives     | 8  |
| <b>CHAPTER TWO</b> |                         |    |
| 2.                 | Materials and methods   | 9  |
| 2.1.               | Materials               | 9  |
| 2.1.1.             | Study design            | 9  |
| 2.1.2.             | Study area              | 9  |
| 2.1.3.             | Study population        | 9  |
| 2.1.4.             | Samples                 | 9  |
| 2.1.5.             | Reagents                | 9  |
| 2.1.6.             | Equipments              | 9  |
| 2.1.7.             | Analysis                | 9  |
| 2.2.               | Methods                 | 10 |



|                      |  |           |
|----------------------|--|-----------|
| <b>CHAPTER THREE</b> |  |           |
| <b>3.</b>            | Results and analysis                       | <b>12</b> |
| <b>CHAPTER FOUR</b>  |  |           |
| <b>4.</b>            | Discussion, Conclusion and Recommendations | <b>18</b> |
| <b>4.1.</b>          | Discussion                                 | <b>18</b> |
| <b>4.2.</b>          | Conclusion                                 | <b>19</b> |
| <b>4.3.</b>          | Recommendations                            | <b>19</b> |
|                      | <b>References</b>                          | <b>20</b> |
|                      | <b>Appendices</b>                          |           |

### List of tables:

| <b>NO</b> | <b>Title</b>   | <b>Pages</b> |
|-----------|--|--------------|
| Table 3-1 | Study group descriptive  | <b>13</b>    |
| Table 3-2 | Study group frequencies  | <b>13</b>    |
| Table 3-3 | Study group frequencies according to sex   | <b>13</b>    |
| Table 3-4 | Independent samples T test for uric acid mean (mg/dl) compare between caraway and uricase methods.     | <b>15</b>    |
| Table 3-5 | Independent samples T test for mean of uric acid (mg/dl) in male compared to female by uricase method. | <b>16</b>    |
| Table 3-6 | Independent samples T test for mean of uric acid (mg/dl) in male compared to female by caraway method. | <b>17</b>    |

### List of figures:

| <b>NO</b>  | <b>Title</b>  | <b>Pages</b> |
|------------|---|--------------|
| Figure 3-1 | Bar chart for study group count according to sex                      | <b>14</b>    |
| Figure 3-2 | Bar chart for compare uric acid in male and female by uricase method. | <b>16</b>    |
| Figure 3-3 | Bar chart for compare uric acid in male and female by caraway method. | <b>17</b>    |

# **CHAPTER ONE**

## **INTRODUCTION AND LITERATURE REVIEW**

## **1. Introduction and Literature Review**

### **1.1. Uric acid:**

#### **1.1.1. Definition :**

Uric acid is the end product of the oxidation purines in the body. It is not only formed from dietary nucleoprotein but also from the break down of cellular nucleoprotein in the body. <sup>(1)</sup>

#### **1.1.2. Biochemistry and physiology of uric acid:**

In the higher primates, such as human and apes, uric acid is the final break down product of purine metabolism. Most other mammals have the ability to catabolize purines one step further to allantoin , a much more water soluble end product. Purine such as adenosine and guanine from the breakdown of ingested nucleic acid or from tissue destruction are converted in to uric acid mainly in the liver. Uric acid is transported by the plasma from the liver to the kidney, where it is filtered by the glomerulus. Reabsorption of 98% to 100% of the uric acid in the glomerular filtrate occurs in the proximal tubules. Small amount of uric acid are then secreted by the distal tubules in to the urine.

Triglyceride, ketone bodies, and lactic acid have been shown to compete with urate for excretory sites in the renal tubules 70% of the daily uric acid excretion the remainder is excreted in to the GIT tract and degraded by bacterial enzymes. Nearly all of uric acid in plasma is present as mono sodium urate. AT pH of plasma urate in this form is relatively insoluble and at levels above 6.4mg/dl the plasma is saturated as a result urate crystals may form and precipitate in the tissue , at PH level <5.7 uric acid is the predominant form and frequently appears as uric acid crystals. <sup>(2)</sup>

Renal handling of uric acid is complex and involve four steps:

Glomerular filtration of virtually all the uric acid in capillary plasma entering the glomerulus, reabsorption in proximal convoluted tubule of about 98% to100% of filtered uric acid, subsequent secretion of uric acid into the lumen in the distal portion of proximal tubule and further reabsorption in the distal. The net urinary excretion of uric acid is 6% to 12% of the amount filtered. <sup>(3)</sup>

### **1.1.3. Disorders of abnormal uric acid:**

#### **1.1.3.1. Genetics:**

Proportion of people have mutations in proteins responsible for the excretion of uric acid by the kidneys. Four genes have so far been identified: SLC22A12, SLC2A9, ABCG2 and SLC17A3. SLC2A9 is known to transport both uric acid and fructose. <sup>(4)</sup>

#### **1.1.3.2. Hyperuricemia:**

Levels High of uric acid is most commonly defined by plasma uric acid concentration greater than 7.0 mg /dL (0.42mmol/L) in male or greater than 6.0 mg/dL (0.36mmol/L) in female. Asymptomatic hyperuricemia is frequently detected through biochemical screening.

Starvation, malnutrition or lead poisoning can increase the amount of uric acid in the blood, also if a joint swollen and painful increased blood uric acid suggest that gout is present. <sup>(5, 3,6,7,8, 9)</sup>

#### **Gout:**

Gout is a common joint disorder, is one of the most controllable metabolic disorder. Occur when uric acid such as monosodium urate accumulates in the blood. This accumulation is due to the inability of kidney to process this normal waste product.

The dysfunction may be due to excess uric acid in the blood, a disorder of purine metabolism or decrease in kidney efficiency and may occur according to person life style. Males contract the disorder after puberty, female after menopause. It is at these time uric acid level increase. If not treated , gout may cause joint deformity.

Death may occur from kidney disease , hypertension, coronary artery disease or stroke. In either case uric acid crystals form and then accumulate in body areas where blood flow is to slow or sluggish to remove the crystals. The elbows, knees and toes are common places of accumulation. If found between the joints, the surrounding tissues becomes inflamed. Nerve endings then become irritated and this causes extreme pain.

Secondary gout is complication of other medical condition such as leukemia and metabolic syndrome. A common diagnosis of gout includes aspiration of synovial fluid from the affected joint or tissue. The fluid is examined by light microscopy for crystals of

monosodium urate intracellular within polymorphonuclear leukocytes. The urate crystal has needle like morphology and strong negative birefringence under polarized light.

Treatment include a change in dietary habits, decreased in ethyl alcohol consumption, and use diuretics and antibiotics. Aspirin should not be taken since like alcohol , it inhibits uric acid secretion.<sup>(3)</sup>

Excess serum accumulation of uric acid in the blood can lead to a type of arthritis known as gout.<sup>(9)</sup> This painful condition is the result of needle-like crystals of uric acid precipitating in joints, capillaries, skin, and other tissues. Kidney stones can also form through the process of formation and deposition of sodium urate microcrystals.<sup>(10)</sup>

Gout can occur where serum uric acid levels are as low as 6 mg/dL (~357  $\mu\text{mol/L}$ ), but an individual can have serum values as high as 9.6 mg/dL (~565  $\mu\text{mol/L}$ ) and not have gout.<sup>(8)</sup>

### **Lesch-Nyhan syndrome:**

Is an x linked disorder and consequently is fully expressed only in affected males. Because of the deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase, there is an inability to salvage the purines, hypoxanthine and guanine and resulting over production of uric acid.<sup>(11)</sup>

Lesch-Nyhan syndrome, an extremely rare inherited disorder, is also associated with very high serum uric acid levels.<sup>(12)</sup> Spasticity, involuntary movement and cognitive retardation as well as manifestations of gout are seen in cases of this syndrome.<sup>(13)</sup>

### **Cardiovascular disease:**

Although uric acid can act as an antioxidant, excess serum accumulation is often associated with cardiovascular disease. It is not known whether this is causative (e.g., by acting as a prooxidant ) or a protective reaction taking advantage of urate's antioxidant properties. The same may account for the putative role of uric acid in the etiology of stroke.<sup>(3)</sup>

### **Type 2 diabetes:**

The association of high serum uric acid with insulin resistance has been known since the early part of the 20th century, nevertheless, recognition of high serum uric acid as a risk factor for diabetes has been a matter of debate. In fact, hyperuricemia has always been presumed to be a consequence of insulin resistance rather than its precursor.<sup>(14)</sup> However, a prospective follow-up study showed high serum uric acid is associated with higher risk of type 2 diabetes, independent of obesity, dyslipidemia, and hypertension.<sup>(15)</sup>

### **Metabolic syndrome:**

Hyperuricemia is associated with components of metabolic syndrome. A study has suggested fructose-induced hyperuricemia may play a pathogenic role in the metabolic syndrome.<sup>(16)</sup> This is consistent with the increased consumption in recent decades of fructose-containing beverages (such as fruit juices and soft drinks sweetened with sugar and high-fructose corn syrup) and the epidemic of diabetes and obesity.<sup>(17)</sup>

### **Uric acid stone formation:**

In normal people, the amount of uric acid excreted in the urine depends on, amongst other things, the purine content of the diet. This makes the definition of a reference range rather difficult, particularly since even at normal excretion rates of 3.6\_4.8mmol/24 h, the urine is super saturated with uric acid and yet most people do not form uric acid stones. Stone formation seems to depend on the presence of hyperuricosuria (more than 6\_7mmol/24h) together with a tendency towards urine with a mean pH that is lower than normal(uric acid is less soluble in acid condition) this offers a useful therapeutic intervention, in that if alkali is used to maintain the uric pH at around 6.5, the formation of stones will be prevented(or even reversed)in mild hyperuricosuria. More pronounced alkalization of the urine should be avoided, as it increases the risk of calcium deposition, patients with severe hyperuricosuria may need allopurinol to prevent stone formation.<sup>(11)</sup>

Saturation levels of uric acid in blood may result in one form of kidney stones when the urate crystallizes in the kidney. These uric acid stones are radiolucent and so do not appear on an abdominal plain X-ray, and thus their presence must be diagnosed by ultrasound for



this reason. Very large stones may be detected on X-ray by their displacement of the surrounding kidney tissues.

Uric acid stones, which form in the absence of secondary causes such as chronic diarrhea, vigorous exercise, dehydration, and animal protein loading, are felt to be secondary to obesity and insulin resistance seen in metabolic syndrome. Increased dietary acid leads to increased endogenous acid production in the liver and muscles, which in turn leads to an increased acid load to the kidneys. This load is handled more poorly because of renal fat infiltration and insulin resistance, which are felt to impair ammonia excretion (a buffer). The urine is therefore quite acidic, and uric acid becomes insoluble, crystallizes and stones form. In addition, naturally present promoter and inhibitor factors may be affected. This explains the high prevalence of uric stones and unusually acidic urine seen in patients with type 2 diabetes. Uric acid crystals can also promote the formation of calcium oxalate stones, acting as "seed crystals" (heterogeneous nucleation).<sup>(18)</sup>

#### **Acute uric acid nephropathy:**

Is disorder in which there is abrupt deterioration in renal function due to the deposition of urate and uric acid crystals within the kidney. It occur predominantly in leukemia of lymphoma patients.<sup>(11)</sup>

#### **1.1.3.3. Hypouricemia:**

Hypouricemia is a level of uric acid in blood serum that is below normal. In humans, the normal range of this blood component has a lower threshold set variously in the range of 2mg/dl to 4mg/dl, while the upper threshold is 530micromol/L (6mg/dL) for women and 619 micromol/L(7mg/dL)for men. Hypouricemia usually is benign and sometimes is a sign of a medical condition.<sup>(19)</sup>

### **Causes:**

Hypouricemia is not a medical condition itself (i.e., it is benign), but it is a useful medical sign. Usually hypouricemia is due to drugs and toxic agents, sometimes it is due to diet or genetics, and rarely it is due to an underlying medical condition. When one of these causal medical conditions is present, hypouricemia is a common sign. <sup>(19)</sup>

### **Multiple sclerosis:**

Lower serum values of uric acid have been associated with multiple sclerosis (MS). MS patients have been found to have serum levels ~194  $\mu\text{mol/L}$ , with patients in relapse averaging ~160  $\mu\text{mol/L}$  and patients in remission averaging ~230  $\mu\text{mol/L}$ . Serum uric acid in healthy controls was ~290  $\mu\text{mol/L}$ . <sup>(20)</sup>

A 1998 study completed a statistical analysis of 20 million patient records, comparing serum uric acid values in patients with gout and patients with multiple sclerosis. Almost no overlap between the groups was found. <sup>(21)</sup>

Uric acid has been successfully used in the treatment and prevention of the animal (murine) model of MS. A 2006 study found elevation of serum uric acid values in multiple sclerosis patients, by oral supplementation with inosine, resulted in lower relapse rates, and no adverse effects. <sup>(22)</sup>

### **Normalizing low uric acid:**

Correcting low or deficient zinc levels can help elevate serum uric acid. <sup>(23)</sup> Inosine can be used to elevate uric acid levels. Zn inhibits Cu absorption, helping to reduce the high Cu/Fe in some people with hypouricemia. Fe supplements can ensure adequate Fe reserves (ferritin above 25 ng/dl), also correcting the high Cu/Fe. <sup>(20)</sup>

### **Oxidative stress:**

Uric acid may be a marker of oxidative stress, <sup>(24)</sup> and may have a potential therapeutic role as an antioxidant. <sup>(25)</sup> On the other hand, like other strong reducing substances such as ascorbate, uric acid can also act as a prooxidant. Thus, it is unclear whether elevated levels of uric acid in diseases associated with oxidative stress such as stroke and atherosclerosis are a protective response or a primary cause. <sup>(26)</sup>

For example, some researchers propose hyperuricemia-induced oxidative stress is a cause of metabolic syndrome.<sup>(16, 27)</sup> On the other hand, plasma uric acid levels correlate with longevity in primates and other mammals.<sup>(28)</sup> This is presumably a function of urate's antioxidant properties.<sup>(29)</sup>

### **Genetics:**

Genetic mutation known to cause hypouricemia are to type:

Xanthine oxidase deficiency, which reduce the production of uric acid and mutation cause abnormal kidney function that increase the excretion of uric acid.<sup>(20, 30, 31)</sup>

### **Diet:**

Hypouricemia is common vegetarians due to the low purine content of most vegetarians diets. Vegetarian diets has been found to result in mean serum uric acid values as low as 239 micromol/L(2.7mg/dL). While avegetarians diet is typically seen as beneficial with respect to condition such as gout, care should be taken to avoid associated health condition.<sup>(5,8)</sup>

### **Medication:**

The majority of drugs that contribute to hypouricemia are uricosurics which is drug that increase the excretion of uric acid from the blood in to the urine.<sup>(32, 33)</sup>

#### **1.1.4. Estimation of uric acid:**

Currently tow methods are widely utilized to quantify uric acid .a colorimetric method depends on the reduction of a chromogen such as sodium tungstate by uric acid to produce amearable colour change. This technique has been commonly employed in automated hospital screening (SMA SYSTEM).the method measures materials other than urate such as ascorbic acid colorimetric determinations are generally considered an overestimation of true uric acid levels, and the normal range is usually 1mg/dl higher than the more specific enzymatic technique. Enzymatic determination of uric acid results from the specific oxidation of uric acid by uricase, which convertes to allantoin. The differential absorbance of these substances at 293nm allows quantification. Although traditionally a more expensive technique, uricase method are currently available for SMA systems at comparable costs and are gradually replacing the less specific colorimetric method.<sup>(34)</sup>

### **1.1.5. Objectives:**

#### **1.1.5.1. General objective:**

To compare between two different methods(chemical and enzymatic) for estimation of serum uric acid.

#### **1.1.5.2. Specific objectives:**

- To estimate uric acid in study group by caraway chemical methods.
- To estimate uric acid in study group by uricase methods.
- To compare uric acid means between the above two methods used for estimation of uric acid.
- To compare uric acid means result between male and female by using uricase enzymatic method and caraway chemical method.

# **CHAPTER TWO**

## **MATERIALS AND METHODS**

## **2. Materials and methods**

### **2.1. Materials**

#### **2.1.1 Study design:**

This is a descriptive analytical comparative study.

#### **2.1.2. Study area:**

The study was conducted in clinical chemistry lab collage of medical laboratories-sudan university.

#### **2.1.3. Study population:**

This study included 50 samples during the period February 2014 – August 2014

#### **2.1.4. Samples:**

Blood samples where used in this study 5.0mL venous blood was collected by standard procedure , from the patient under study. They were taken into sterile containers with heparin as anticoagulant to measure the uric acid.

#### **2.1.5. Reagents:**

##### **Uricase enzyme method:**

These reagents produced by crescent diagnostic company and includes: uricase enzyme, peroxidase, 4-amino antipyrine , phenol and phosphate buffer.

##### **Phospotungstic acid (PTA) method:**

**R1:**5%sodium tungstate.

**R2:**2/3N sulphuric acid.

**R3:**10%sodium carbonate.

**R4:** phosphotungstic acid.

#### **2.1.6. Equipment:**

Colorimeter was used in this study.

#### **2.1.7. Analysis:**

Data was analyzed by using the computer (SPSS) programme.

Colorimeter was used in this study.

### **2.2. Methodology:**

#### **(1).Estimation of uric acid by uricase enzyme method:**

**Principle of method:**

Uric acid is oxidized by uricase to give allantoin & hydrogen peroxide, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) reduced by peroxidase. Oxygen of peroxidase accepted by 4-amino antipyrine which oxidize phenol to give pink quinonimine, measured colorimetrically at 520 nm. <sup>(35)</sup>

**Procedure:**

Three labeled test tubes was prepared as follow:

|                    | <b>B</b> | <b>S</b> | <b>T</b> |
|--------------------|----------|----------|----------|
| <b>W.R</b>         | 1.0      | 1.0      | 1.0mL    |
| <b>STD(6mg/dl)</b> | –        | 0.025mL  | –        |
| <b>Sample</b>      | –        | –        | 0.025mL  |

The tubes were incubated for 10 minutes at room temperature. then the absorbance read at 520nm against reagent blank.

**Calculation:**

$$\frac{(A) \text{ Sample}}{(A) \text{ standard}} \times 6(\text{standard conc}) = \text{mg/dL uric acid in the sample}$$

**Quality control:**

Control sera are recommended to monitor the performance of assay procedure :

SPINTROL H Normal and Pathologic (Ref.1002120 and 1002210).

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own quality control scheme and corrective actions if controls do not meet the acceptable tolerances.

**Reference values:**

Women : 2.5-6.8 mg/dL

Men : 3.6-7.7 mg/dL

**(2).Estimation of uric acid by caraway method:**

**Principle of method:**

Uric acid reduced phosphotungstic acid to tungsten blue in an alkaline medium. The intensity of the colour produced is proportional to the amount of the uric acid present in the reaction mixture.<sup>(3)</sup>

**Procedure:**

Labeled centrifuge test tube was prepared as follow:

|        |       |
|--------|-------|
| DW     | 0.5Ml |
| Sample | 0.5mL |
| R1     | 0.5mL |
| R2     | 0.5mL |

The tube was mixed and centrifuged at 3000 rpm for 5 minutes, then continued as follow:

Three labeled test tubes were prepared as follow :

|               | B     | S     | T     |
|---------------|-------|-------|-------|
| Supernatant   | –     | –     | 0.9Ml |
| STD(0.5mg/dl) | –     | 0.9mL | –     |
| D.W           | 0.9mL | –     | –     |
| R3            | 0.3mL | 0.3mL | 0.3Ml |
| R4            | 0.3mL | 0.3mL | 0.3Ml |

The tubes were incubated for 15 minutes at room temperature (RT).Then the absorbances is read at 710nm against reagent blank.

**Calculation:**

$$\frac{(A) \text{ Sample}}{(A) \text{ standard}} \times 0.5(\text{standard conc}) \times 4 (\text{D.F}) = \text{mg/dL uric acid in the sample}$$



# **CHAPTER THREE**

## **RESULTS**

### 3. Results

**Table (3-1):** Study group descriptive.

**Table (3-2):** Study group frequencies.

**Table (3-3):** Study group frequencies according to sex.

**Table (3-4):** Independent samples T test for uric acid mean (mg/dl) compare between caraway and uricase methods.

**Table (3-5):** Independent samples T test for mean of uric acid (mg/dl) in male compared to female by uricase method.

**Table (3-6):** Independent samples T test for mean of uric acid (mg/dl) in male compared to female by caraway method.

**Figure (3-1):** Bar chart for study group count according to sex.

**Figure (3-2):** Bar chart for compare uric acid in male and female by uricase method.

**Figure (3-3) :** Bar chart for compare uric acid in male and female by caraway method.

**Table (3 - 1): Study group descriptive.**

**Descriptive Statistics**

|                     | N  | Minimum | Maximum | Mean  | Std. Deviation |
|---------------------|----|---------|---------|-------|----------------|
| CARAWY (U.A mg/dL)  | 50 | .7      | 6.4     | 3.326 | 1.1206         |
| URICACE (U.A mg/dL) | 50 | 1.8     | 7.8     | 3.854 | 1.2301         |
| SEX                 | 50 | 1       | 2       | 1.52  | .505           |
| Valid N (listwise)  | 50 |         |         |       |                |

**Table (3 - 2): Study group frequencies.**

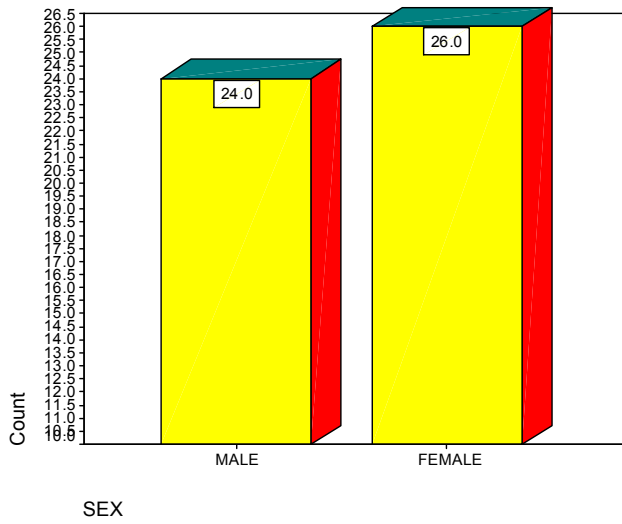
**Statistics**

|   |         | CARAWY<br>(U.A mg/dL) | URICACE<br>(U.A mg/dL) | SEX |
|---|---------|-----------------------|------------------------|-----|
| N | Valid   | 50                    | 50                     | 50  |
|   | Missing | 0                     | 0                      | 0   |

**Table (3 - 3): Study group frequencies according to sex.**

**SEX**

|       |        | Frequency | Percent | Valid Percent | Cumulative<br>Percent |
|-------|--------|-----------|---------|---------------|-----------------------|
| Valid | MALE   | 24        | 48.0    | 48.0          | 48.0                  |
|       | FEMALE | 26        | 52.0    | 52.0          | 100.0                 |
| Total |        | 50        | 100.0   | 100.0         |                       |



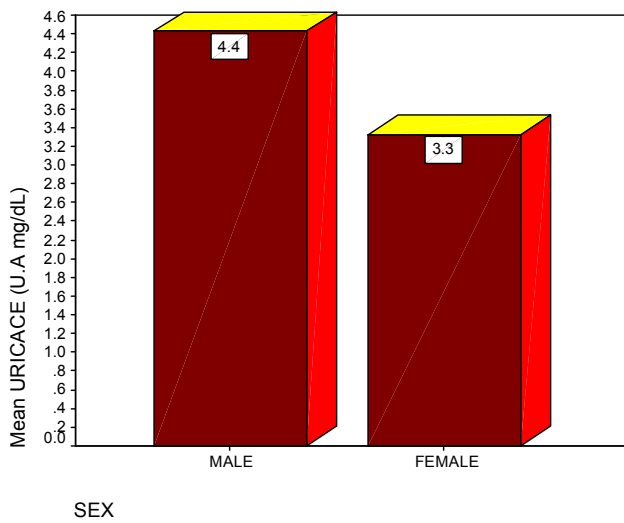
**Figure (3 - 1): Bar chart for study group count according to sex.**

**Table (3 - 4):** Independent samples T test for uric acid mean compare between caraway and uricase methods.

| <b>Method</b>  | <b>No</b> | <b>Mean (mg/dL)</b> | <b>ST.D (mg/dL)</b> | <b>P. Value</b> |
|----------------|-----------|---------------------|---------------------|-----------------|
| <b>Caraway</b> | 50        | 3.3                 | 1.1                 | 0.027           |
| <b>Uricase</b> | 50        | 3.9                 | 1.2                 |                 |

**Table (3 - 5):** Independent samples T test for mean of uric acid ( mg/dl) in male compared to female by uricase method.

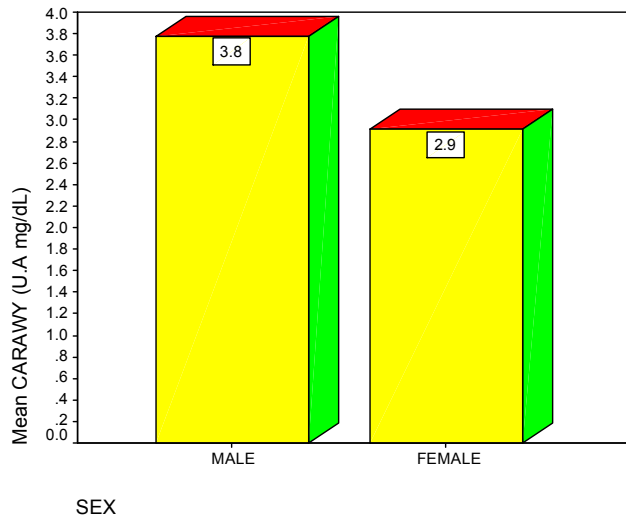
| Sex    | No | Mean (mg/dL) | ST.D (mg/dL) | P. Value |
|--------|----|--------------|--------------|----------|
| Male   | 24 | 4.4          | 1.3          | 0.001    |
| Female | 26 | 3.3          | 0.8          |          |



**Figure (3 - 2):** Bar chart for compare uric acid in male and female by uricase method.

**Table (3 - 6):** Independent samples T test for mean of uric acid ( mg/dl) in male compared to female by caraway method.

| Sex    | No | Mean (mg/dL) | ST.D (mg/dL) | P. Value |
|--------|----|--------------|--------------|----------|
| Male   | 24 | 3.8          | 1.3          | 0.005    |
| Female | 26 | 2.9          | 0.8          |          |



**Figure (3 - 3):** Bar chart for compare uric acid in male and female by caraway method.

# **CHAPTER FOUR**

## **DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS**



## 4. Discussion, Conclusions and Recommendations

### 4.1. Discussion:

This study was conducted in the Sudan university (SUST) during February 2014 – August 2014 , in this study plasma collected from each one of 50 volunteers by standard procedure (24 male and 26 female ) and aimed to compare between the plasma uric acid by the uricase enzymatic method and by caraway chemical method.

Uric acid levels were estimate in all sample using caraway and uricase methods and all analyzed by using SPSS program (P value =0.027) AS indicated in table(3-4 ) there was statistical significant increase in uric acid mean in uricase enzymatic method ( $3.9 \pm 1.2$  mg/dl) than caraway chemical method ( $3.3 \pm 1.1$ mg/dl) with (p. value = 0 .027) as in table(3-4) This results was disagree to previous study (serum uric acid estimation chemical and enzymatic method compared) done by M.J. Buchanan, I.C. Isdale ,and B .S. Rose , Which reveal that in level below 6 mg/dl, chemical method had higher reading than uricase enzymatic method and that the

(mean =  $4 \pm 1.4$  mg/dl) of serum uric acid level was insignificantly lower than that of chemical method (mean =  $4.6 \pm 0.56$  mg /dl).<sup>(36)</sup> This disagree may be due to small samples size or not used quality control program.

As indicate in table (3-5) there was statistical significant increase in uric acid levels in males (mean=  $4.4 \pm 1.3$ mg/dl) than female (mean= $3.3 \pm 0.8$ mg/dl) by uricase enzymatic method, and also there was statistical significant increase in uric acid levels in males (mean=  $3.8 \pm 1.3$ mg/dl) than female(mean=  $2.9 \pm 0.8$ mg/dl) by caraway chemical method, indicate in table (3-6 ).

This results was agree to previous study (Serum uric acid estimation chemical and enzymatic methods compared) done by M.J. Buchanan, I.C. Isdale , and B .S. Rose , Which reveal that the uric acid levels tending to be lower in fourteen women than the thirteen men of the samples.<sup>(36)</sup>

## **4.2. Conclusions:**

From the results of this study it is concludes that:

- There was statistical significant increase in uric acid mean in uricase method than caraway method.
- There was statistical significant increase in uric acid mean in males than females.
- The Enzymatic advantages of being less time consuming, easy to perform and less costly.
- The commercially available method for measurement uric acid was enzymatic method.
- In contrast caraway method was time consuming, more expensive and complicated.

## **4.3. Recommendations:**

From the results of this study it is recommended that:

- The enzymatic and chemical method can be used in Sudan for uric acid measurement considering the reference value of each one by using quality control program.
- When estimating uric acid sex (male or female) must be considerable in reference values.
- Also, we recommended further studies to confirm the result of this study.
- Increase samples size to get more accurate results.

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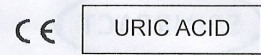
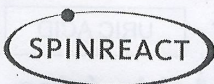
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# APPENDICES



# Uric acid

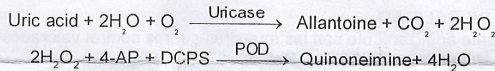
Uricase -POD. Enzymatic colorimetric

## Quantitative determination of uric acid IVD

Store at 2-8°C

### PRINCIPLE OF THE METHOD

Uric acid is oxidized by uricase to allantoin and hydrogen peroxide (2H<sub>2</sub>O<sub>2</sub>), which under the influence of POD, 4-aminophenazone (4-AP) and 2-4 Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:



The intensity of the red color formed is proportional to the uric acid concentration in the sample<sup>1,2</sup>.

### CLINICAL SIGNIFICANCE

Uric acid and its salts are end products of the purine metabolism. With progressive renal insufficiency, there is retention in blood of urea, creatinine and uric acid.

Elevate uric acid level may be indicative of renal insufficiency and is commonly associated with gout<sup>1,5,6</sup>.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

### REAGENTS

|               |                                     |           |
|---------------|-------------------------------------|-----------|
| R 1           | Phosphate pH 7.4                    | 50 mmol/L |
| Buffer        | 2-4 Dichlorophenol sulfonate (DCPS) | 4 mmol/L  |
| R 2           | Uricase                             | 60 U/L    |
| Enzymes       | Peroxidase (POD)                    | 660 U/L   |
|               | Ascorbate oxidase                   | 200 U/L   |
|               | 4 - Aminophenazone (4-AP)           | 1 mmol/L  |
| URIC ACID CAL | Uric acid aqueous primary standard  | 6 mg/dL   |

### PREPARATION

Working reagent (WR): Dissolve (→) the contents of one vial R 2 Enzymes in one bottle R 1 Buffer. Cap and mix gently to dissolve contents. (WR) is stable after reconstitution 1 month at 2-8°C or 10 days at room temperature.

### STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

### Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 520 nm ≥ 0.16.

### ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 520 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

### SAMPLES

- Serum or plasma<sup>1</sup>: Stability 3-5 days at 2-8°C or 6 months at -20°C.
- Urine (24 h)<sup>1</sup>: Stability 4 days at 15-25°C, pH >8. Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor); If urine is cloudy; warm the specimen to 60°C for 10 min to dissolve precipitated urates and uric acid. Do not refrigerate.

### PROCEDURE

- Assay conditions:  
Wavelength: ..... 520 nm (490-550)  
Cuvette: ..... 1 cm light path  
Temperature ..... 37°C / 15-25°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

|                                     | Blank | Standard | Sample |
|-------------------------------------|-------|----------|--------|
| WR (mL)                             | 1.0   | 1.0      | 1.0    |
| Standard <sup>(Note 1-2)</sup> (μL) | --    | 25       | --     |
| Sample (μL)                         | --    | --       | 25     |

- Mix and incubate for 5 min at 37°C or 10 min at 15-25°C.
- Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

### CALCULATIONS

Serum or plasma

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 6 \text{ (Standard conc.)} = \text{mg/dL uric acid in the sample}$$

Urine 24 h

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 6 \times \text{vol. (dL) urine 24 h} = \text{mg/24 h uric acid}$$

Conversion factor: mg/dL x 59.5 = μmol/L.

### QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

### REFERENCE VALUES<sup>5</sup>

Serum or plasma:

Women 2.5 - 6.8 mg/dL ≅ 149 - 405 μmol/L

Men 3.6 - 7.7 mg/dL ≅ 214 - 458 μmol/L

Urine: 250 - 750 mg/24 h ≅ 1.49 - 4.5 mmol/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

### PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.03 mg/dL to linearity limit of 25 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

### Precision:

| Mean (mg/L) | Intra-assay (n=20) |      | Inter-assay (n=20) |      |
|-------------|--------------------|------|--------------------|------|
|             | 4.74               | 11.4 | 4.72               | 11.2 |
| SD          | 0.03               | 0.06 | 0.07               | 0.15 |
| CV (%)      | 0.63               | 0.56 | 1.58               | 1.36 |

Sensitivity: 1 mg/dL = 0.0347 A.

Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.99.

Regression equation: y=1.005x + 0.0005.

The results of the performance characteristics depend on the analyzer used.

### INTERFERENCES

No interferences were observed to bilirubin up to 170 μmol/L, hemoglobin up to 130 mg/dL and ascorbic acid up to 570 μmol/L<sup>2</sup>.

A list of drugs and other interfering substances with uric acid determination has been reported by Young et al.<sup>3,4</sup>.

### NOTES

- URIC ACID CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.
- SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

### BIBLIOGRAPHY

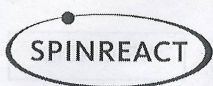
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### PACKAGING

|              |      |  |
|--------------|------|--|
| Ref: 1001010 | Cont | R1: 10 x 20 mL, R2:10 → 20 mL, CAL: 1 x 5 mL |
| Ref: 1001011 |      | R1: 10 x 50 mL, R2:10 → 50 mL, CAL: 1 x 5 mL |
| Ref: 1001012 |      | R1: 4 x 125 mL, R2:4 → 125mL, CAL: 1 x 5 mL  |
| Ref: 1001013 |      | R1: 4 x 250 mL, R2:4 → 250mL, CAL: 1 x 5 mL  |







CE URIC ACID

# Ácido úrico

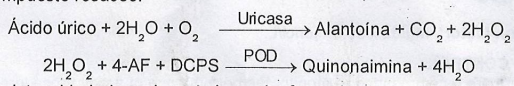
Uricasa -POD. Enzimático colorimétrico

## Determinación cuantitativa de ácido úrico IVD

Conservar a 2-8°C

### PRINCIPIO DEL MÉTODO

El ácido úrico es oxidado por la uricasa a alantoína y peróxido de hidrógeno (2H<sub>2</sub>O<sub>2</sub>) que en presencia de peroxidasa (POD), 4-aminofenazona (4-AF) y 2-4 Diclorofenol Sulfonato (DCPS) forma un compuesto rosáceo:



La intensidad de quinonaimina roja formada es proporcional a la concentración de ácido úrico presente en la muestra ensayada<sup>1,2</sup>.

### SIGNIFICADO CLÍNICO

El ácido úrico y sus sales son el producto final del metabolismo de las purinas. En una insuficiencia renal progresiva hay una retención en sangre de urea, creatinina y ácido úrico. Niveles altos de ácido úrico son indicativos de patología renal y generalmente se asocia con la gota<sup>1,5,6</sup>. El diagnóstico clínico debe realizarse teniendo en cuenta todos los datos clínicos y de laboratorio.

### REACTIVOS

|               |                                       |           |
|---------------|---------------------------------------|-----------|
| R 1           | Fosfatos pH 7,4                       | 50 mmol/L |
| Tampón        | 2-4 Diclorofenol Sulfonato (DCPS)     | 4 mmol/L  |
| R 2           | Uricasa                               | 60 U/L    |
| Enzimas       | Peroxidasa (POD)                      | 660 U/L   |
|               | Ascorbato oxidasa                     | 200 U/L   |
|               | 4 - Aminofenazona (4-AF)              | 1 mmol/L  |
| URIC ACID CAL | Patrón primario acuoso de Ácido úrico | 6 mg/dL   |

### PREPARACIÓN

Reactivo de trabajo (RT): Disolver (→) el contenido de un vial de R 2 Enzimas en un frasco de R 1 Tampón. Tapar y mezclar suavemente hasta disolver su contenido. Estabilidad: 1 mes en nevera (2-8°C) o 10 días a temperatura ambiente.

### CONSERVACIÓN Y ESTABILIDAD

Todos los componentes del kit son estables, hasta la fecha de caducidad indicada en la etiqueta, cuando se mantienen los frascos bien cerrados a 2-8°C, protegidos de la luz y se evita su contaminación. No usar reactivos fuera de la fecha indicada.

### Indicadores de deterioro de los reactivos:

- Presencia de partículas y turbidez.
- Absorbancia (A) del Blanco a 520 nm ≥ 0,16.

### MATERIAL ADICIONAL

- Espectrofotómetro o analizador para lecturas a 520 nm.
- Cubetas de 1,0 cm de paso de luz.
- Equipamiento habitual de laboratorio.

### MUESTRAS

- Suero o plasma<sup>1</sup>: Estabilidad 3-5 días a 2-8°C y 6 meses a -20°C.
- Orina (24 h)<sup>1</sup>: Estabilidad 3 días a temperatura ambiente a pH > 8. Diluir la muestra al 1/50 en agua destilada. Mezclar. Multiplicar el resultado obtenido por 50 (factor de dilución); Si la muestra es turbia, calentarla a 60°C 10 min para disolver los precipitados de urato y ácido úrico. No refrigerar.

### PROCEDIMIENTO

- Condiciones del ensayo:
  - Longitud de onda: ..... 520 nm (490-550)
  - Cubeta: ..... 1 cm paso de luz
  - Temperatura ..... 37°C / 15-25°C
- Ajustar el espectrofotómetro a cero frente a agua destilada.
- Pipetear en una cubeta:

|                        | Blanco | Patrón | Muestra |
|------------------------|--------|--------|---------|
| RT (mL)                | 1,0    | 1,0    | 1,0     |
| Patrón (Nota 1-2) (µL) | --     | 25     | --      |
| Muestra (µL)           | --     | --     | 25      |

- Mezclar e incubar 5 minutos a 37°C ó 10 min. 15-25°C.
- Leer la absorbancia (A) del Patrón y la muestra, frente al Blanco de reactivo. El color es estable como mínimo 30 minutos.

### CÁLCULOS

Suero o plasma

$$\frac{(A) \text{Muestra}}{(A) \text{Patrón}} \times 6 \text{ (Conc. Patrón)} = \text{mg/dL de ácido úrico en la muestra}$$

Orina 24 h

$$\frac{(A) \text{Muestra}}{(A) \text{Patrón}} \times 6 \times \text{vol. (dL) orina/24h} = \text{mg/24 h de ácido úrico}$$

Factor de conversión: mg/dL x 59,5= µmol/L.

### CONTROL DE CALIDAD

Es conveniente analizar junto con las muestras sueros control valorados: CONTROL H Normal y Patológico (Ref. 1002120 y 1002210). Si los valores hallados se encuentran fuera del rango de tolerancia, revisar el instrumento, los reactivos y el calibrador. Cada laboratorio debe disponer su propio Control de Calidad y establecer correcciones en el caso de que los controles no cumplan con las tolerancias.

### VALORES DE REFERENCIA<sup>5</sup>

Suero o plasma:

- Mujeres 2,5 - 6,8 mg/dL ≅ 149 - 405 µmol/L
- Hombres 3,6 - 7,7 mg/dL ≅ 214 - 458 µmol/L

Orina:

250 - 750 mg/24 h ≅ 1,49 - 4,5 mmol/24 h

Estos valores son orientativos. Es recomendable que cada laboratorio establezca sus propios valores de referencia.

### CARACTERÍSTICAS DEL MÉTODO

Rango de medida: Desde el límite de detección de 0,03 mg/dL hasta el límite de linealidad de 25 mg/dL.

Si la concentración es superior al límite de linealidad, diluir la muestra 1/2 con ClNa 9 g/L y multiplicar el resultado final por 2.

### Precisión:

|              | Intraserie (n= 20) |      | Interserie (n= 20) |      |
|--------------|--------------------|------|--------------------|------|
| Media (mg/L) | 4,74               | 11,4 | 4,72               | 11,2 |
| SD           | 0,03               | 0,06 | 0,07               | 0,15 |
| CV (%)       | 0,63               | 0,56 | 1,58               | 1,36 |

Sensibilidad analítica: 1 mg/dL = 0,0347 A.

Exactitud: Los reactivos SPINREACT (y) no muestran diferencias sistemáticas significativas cuando se comparan con otros reactivos comerciales (x).

Los resultados obtenidos con 50 muestras fueron los siguientes:

Coefficiente de correlación (r): 0,99.

Ecuación de la recta de regresión: y=1,005x + 0,0005.

Las características del método pueden variar según el analizador utilizado.

### INTERFERENCIAS

No se han observado interferencias con bilirrubina hasta 170 µmol/L, hemoglobina hasta 130 mg/dL y ácido ascórbico hasta 570 µmol/L<sup>2</sup>.

Se han descrito varias drogas y otras sustancias que interfieren en la determinación del ácido úrico<sup>3,4</sup>.

### NOTAS

- URIC ACID CAL: Debido a la naturaleza del producto, es aconsejable tratarlo con sumo cuidado ya que se puede contaminar con facilidad.
- La calibración con el Patrón acuoso puede dar lugar a errores sistemáticos en métodos automáticos. En este caso, se recomienda utilizar calibradores séricos.
- Usar puntas de pipeta desechables limpias para su dispensación.
- SPINREACT dispone de instrucciones detalladas para la aplicación de este reactivo en distintos analizadores.

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### PRESENTACIÓN

|              |       |  |
|--------------|-------|--|
| Ref: 1001010 | Cont. | R1: 10 x 20 mL, R2:10 → 20 mL, CAL: 1 x 5 mL |
| Ref: 1001011 |       | R1: 10 x 50 mL, R2:10 → 50 mL, CAL: 1 x 5 mL |
| Ref: 1001012 |       | R1: 4 x 125 mL, R2:4 → 125mL, CAL: 1 x 5 mL  |
| Ref: 1001013 |       | R1: 4 x 250 mL, R2:4 → 250mL, CAL: 1 x 5 mL  |