**Intended use**. System for creatinine determination in serum, plasma, urine and amniotic liquid by two point kinetic method.

**Professional use.**

**Test principle**. Creatinine reacts to alkaline picrate yielding a red complex. The amount of the color is proportional to creatinine concentration (not corrected) in the sample.

\[
\text{Creatinine} + \text{Alkaline picrate} \rightarrow \text{Creatinine picrate}
\]

**Summary**. Creatinine K VET applies a two point optimized procedure in order to improve the method specificity and minimize the susceptibility to interferences\(^1\)-\(^3\).

The measurement procedure is calibrated with the NIST SRM 914 and renders the results traceable to the IDMS (isotropic dilution, mass spectrometry) definitive method, which complies the National Kidney Disease Education Program (NKDEP) recommendations for standardization of serum creatinine measurement\(^4\).

All the methods that apply the Jaffe reaction are susceptible to a constant systematic error, due to the plasmatic proteins and other chromogens interference. In order to minimize this error and increase the Creatinine K VET results accuracy, Labtest recommends the use of the correction index\(^5\),\(^12\) (see Calculation) that must be applied whatever are the found results.

Creatinine K VET presents a sample treatment procedure with ferricyanide\(^6\),\(^7\) that oxidizes the present bilirubin (in concentration of 5.0 up to 19 mg/dL) and excludes its negative interference, while the desproteinization procedure removes interference of lipemic samples equivalent to a triglycerides value of 900 up to 1800 mg/dL\(^12\). This method was based on the performed studies that demonstrated that is possible minimize significantly the interferences caused by icteric and lipemic samples in creatinine measurement\(^3\).

The alkaline picrate keeps the analytic performance when tightly closed at 2 - 8 °C for 15 days, allowing the preparation of a high volume of working reagent according to the laboratory routine.

The measurement procedure is applied in automated and semi-automated systems able to perform accurate measure of absorbance at 510 nm.

**Methodology**. Labtest

**Reagents**

1. **REAGENT 1** - NaOH - Store at 15 - 30 °C.
   Reagent label bears expiration date. Sodium hydroxide (200 mmol/L).
   Corrosive reagent.

2. **REAGENT 2** - Picric Acid - Store at 15 - 30 °C.
   Reagent label bears expiration date. Picric acid (22.2 mmol/L).

3. **STANDARD** - Standard 4.0 mg/dL - Store at 2 - 30 °C.
   Reagent label bears expiration date. Creatinine 4.0 mg/dL.

4. **REAGENT 4** - Ferricyanide - Store at 15 - 30 °C.
   Potassium ferricyanide (11 mmol/L). Do not keep in the refrigerator.

**Precautions and warnings**

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

The NaOH (\(^\ast\) 1) is corrosive and may result in skin and eyes burns and irritations and ulcerations when ingested. In case of ingestion, immediately ingest a lot of water with lemon juice or vinegar. Do not induce vomiting and get medical assistance. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.

In case of ingestion of Picric Acid (\(^\ast\) 2), offer 4 glasses of water and if the individual is conscious, induce vomiting and get medical assistance.

In case of ingestion of Ferricyanide (\(^\ast\) 4), offer 4 glasses of water and if the individual is conscious, induce vomiting and get medical assistance.

**Storage and stability**. Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. In order to avoid evaporation of the Standard, keep the bottle tightly closed.

**Deterioration**. Microbial or chemical contamination may decrease reagents stability. Alkaline Picrate is not suitable for use if it has an absorbance over 0.200 at 510 nm when measured versus water as reference.

**Pre-analytical influences**. For therapeutic control, it is suggested to collect sample at the same time due to circadian changes of the creatinine.

It is important to note that creatinine is more labile than most substrates. As a result, fresh samples should be prioritized. The results of samples collected several days ago may not be accurate.
The creatinine concentration is not affected by diet or any other factor that affects the hepatic metabolism and cycle of urine. After absorption of nutrients, high protein diets increases serum creatinine concentration, which is compensated by an increase in postprandial GFR.

Intense exercises can raise creatinine values.

Cephalosporin antibiotics may interfere the tests.

**Specimen collection and preparation**

Use serum or plasma (fluoride, heparin, EDTA, oxalate and citrate). Creatinine is reportedly stable for about 7 days at 2 - 8 ºC. The use of the anticoagulant Glistab VET (Labtest Ref.: 1016) allows the collection of only one sample to the urea, glucose and creatinine measurements.

24 hours urine and amniotic liquid must be centrifuged. The urine sample should not receive preservatives and must be kept at 2 - 8 ºC during the collection period and after the delivery in the laboratory.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

**Interference**

Proteins present in the samples yield a positive interference introducing a constant systematic error. This error may be minimized applying a correction index. Since the urine has no proteins that may interfere, the correction index is not applied to the calculation of concentration in samples of urine. See applications of correction index on Calculation.

Creatinine determination in urine may be affected by the action of high amount of reducers substances present in cases of ketoacidosis. Boiling the urine sample for one minute eliminates partially these substances interference. The remaining interference is excluded in the kinetic measurement.

Bilirubin over 5 mg/dL interferes negatively in the reaction. Hemoglobin up to 180 mg/dL and triglycerides up to 900 mg/dL do not interfere in the reaction.

**Eliminating the interferences action**. Samples with bilirubin concentration of 5 to 19 mg/dL, the interference may be eliminated by the following procedure: add 0.05 mL of Ferricyanide (Nº 4) to 0.5 mL of the sample. Mix and wait 5 minutes. Determine the creatinine, multiply the result by 1.1 and apply the correction index.

If bilirubin concentration is over 19 mg/dL and lower than 38 mg/dL, dilute the sample 1:2 with 0.85% NaCl (150 mmol/L). Add 0.05 mL of Ferricyanide (Nº 4) to 0.5 mL of the diluted sample. Mix and wait 5 minutes. Determine the creatinine, multiply the result by 2.2 and apply the correction index.

If triglycerides concentrations are ranging from 900 mg/dL and 1800 mg/dL the interference due the lipemic sample may be eliminated by the desproteinization procedure. If triglycerides concentrations are over 1800 mg/dL and lower than 3500 mg/dL, dilute the sample 1:2 with 0.85% NaCl (150 mmol/L), and follow the procedures with desproteinization for creatinine determination and multiply the result by 2.

Do not apply the correction index when using the desproteinization procedure.

Dilutions over 1:2 are not recommended because, in samples with low creatinine concentrations, it is obtained results with significant errors due the increasing of the analytical imprecision.

**Materials required not provided**

1. Photometer with cuvette at 37 ºC capable of measuring absorbance in kinetic method.
2. Automated equipment able to process one or two reagents (for automated applications).
3. Pipettes to measure reagents and samples.
4. Timer (for manual applications).

**Preparing the reagents**

Mix 4 volumes of NaOH (Nº 1) with 1 volume of Picric Acid (Nº 2). Stable 15 days at 2 - 8 ºC in a plastic bottle tightly closed. Deterioration is indicated by an absorbance over 0.200 if Alkaline Picrate is measured against water at 510 nm.

Atmospheric CO$_2$ alters significantly the NaOH (Nº 1) and Alkaline Picrate stability, if kept in open bottles. The stability modification is influenced by exposure time and environment conditions. It is suggested to keep in the equipment only the volume enough to the samples measurement or using the information of Quality Control as indicator for performing new calibration.

**Manual procedure**

Urine: dilute the sample 1:25 (0.2 mL of urine + 4.8 mL of distilled or deionized water). Multiply the result by 25.

Temperature control is absolutely important for reproducibility of results. Since the time of reaction is very small, it is necessary to use equipment with cuvette at 37 ºC.

It is fundamental that the operations with samples and standards are performed always in the same way, keeping constant the time interval between the sample or standard mixture with the reagent and the beginning of the photometric measurement.

In case of the proposed volumes are not enough to the equipment for photometric readings, increase proportionally the volumes of Alkaline Picrate and sample or Standard.

**Direct procedure**. Zero the photometer at 510 nm (490 to 520 nm) with water. Add 0.10 mL of Standard or sample to 1.0 mL of Alkaline Picrate. Mix and insert into the cuvette. Start the timer and measure the absorbance after 30 and 90 seconds.

**Procedure with desproteinization**. Mix 0.2 mL of serum to 0.4 mL of Picric Acid (Nº 2), mix and centrifuge during 10 minutes. Adjust the zero of photometer at 510 nm (490 to 520 nm) with water. In other tube, pipette 0.8 mL of NaOH (Nº 1) and add 0.3 mL of the clear supernatant. Mix and insert into the cuvette. Start the timer and measure the absorbance after 30 and 90 seconds. The Standard should be measured using the direct procedure. Do not apply the correction index.
Endogenous creatinine depuration. The patient should receive explanations in order to collect the 24 hours urine correctly.

Measure the serum and urine creatinine using the proposed methodologies. Serum may be obtained in any moment during the urine collection.

Apply the results obtained in the following equation:

\[
\text{Depuration} = \frac{U}{S} \times VM \text{ (mL/ minute)}
\]

where:
- \(U\) = creatinine in the urine (mg/dL)
- \(S\) = corrected creatinine in the serum (mg/dL)
- \(VM\) = Volume per minute (urinary volume of 24 hours, in mL, divided by 1440).

**PS:** Depuration must be corrected for the body surface of the patient that is obtained by a nomogram correlating weight and height, or using the following equation:

\[
A = W^{0.425} \times H^{0.725} \times 0.007184
\]

where:
- \(W\) = weight (kg)
- \(H\) = height (cm)

Multiply the depuration value by 1.73 and divide by the patient body surface.

Glomerular filtration rate. The NKDEP recommends that the laboratories report the estimated glomerular filtration rate (eGFR) in all the reports containing creatinine results.

Normal values of creatinine are found in healthy animals, in a wide range of variation, make difficult to establish an accurate reference range for the analyte.

Some researchers believe that the production of creatinine is proportional to the human muscle mass. According to them, even within a given species, an individual of greater muscle mass can have a value slightly above the normal limit of the reference value.

In general, the normal plasma concentration is less than 150 μmol/L (1.6 mg/dL), varying between species.

### On average, the following values are accepted (mg/dL)

<table>
<thead>
<tr>
<th>Species</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Canidae</td>
<td>0.5 - 1.5</td>
</tr>
<tr>
<td>Felidae</td>
<td>0.7 - 1.8</td>
</tr>
<tr>
<td>Equidae</td>
<td>1.2 - 1.9</td>
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<tr>
<td>Bovinae</td>
<td>1.0 - 1.8</td>
</tr>
<tr>
<td>Caprinae</td>
<td>0.6 - 1.4</td>
</tr>
<tr>
<td>Ovis</td>
<td>0.6 - 1.4</td>
</tr>
<tr>
<td>Suidae</td>
<td>0.5 - 2.1</td>
</tr>
</tbody>
</table>
Factors such as cytokines that cause an increase in endogenous muscle catabolism in cachexia caused by cancer or sepsis may increase the release of creatine and, consequently, production of creatinine.

Small increases are usually associated with pre-renal factors (heart failure or dehydration) while relatively large increases tend to be related to renal factors.

Values greater than 5 mg/dL may indicate renal failure. Concentrations greater than 10 mg/dL are found in severe ARF, final stages of CRD and ruptured bladder and urethral obstruction.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.
2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity 1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be <0.1 mg/L.
3. It is suggested to consult www.fxol.org in order to review physiopathological source and drugs interference in results and methodology.

References


Performance characteristics

Recovery studies. In two samples with creatinine concentrations of 2.7 and 8.7 mg/dL were added different quantities of the analyte. Subsequent analyses provided recoveries ranging from 97 to 100%. The mean proportional systematic error at 1.6 mg/dL decision level was 0.0232 mg/dL or 1.45%.

Method comparison. A group of 20 sera were assayed by the proposed method and an enzymatic method (traceable to IDMS method). Serum creatinine values ranged from 0.52 - 11.0 mg/dL. The comparisons yielded a correlation coefficient of 0.998 and regression equation was y = 1.0073x + 0.0281. The mean total systematic error (proportional and constant) at 1.00 mg/dL, 1.20 mg/dL and 2.00 mg/dL decision levels were 0.035 mg/dL (3.53%), 0.037 mg/dL (3.07%) and 0.043 mg/dL (2.13%), respectively.

Imprecision - Within Run

<table>
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<tr>
<th></th>
<th>N</th>
<th>Mean (mg/dL)</th>
<th>SD (mg/dL)</th>
<th>(%CV)</th>
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<tbody>
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<td>0.66</td>
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Imprecision - Run-to-Run

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<th>(%CV)</th>
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<tr>
<td>Sample 3</td>
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<td>7.49</td>
<td>0.273</td>
<td>3.64</td>
</tr>
</tbody>
</table>

Analytical sensitivity. Detection limit: 0.14 mg/dL. The detection limit represents the lowest measurable creatinine concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without creatinine.

Clinical meaning. Like urea, plasma creatinine is used to investigate renal disease. Evaluation of these substances provides a greater number of clinical information.

The consistency in formation and excretion makes creatinine a very useful marker of renal function, especially of glomerular filtration due to its relative independence of factors such as diet, hydration degree and protein metabolism. Thus, plasma creatinine clearance is a marker of safer renal function than urea.

Creatinine should not be used alone to assess the rate of glomerular filtration or to detect the presence of chronic kidney disease because it is affected by glomerular filtration rate and by independent factors such as age, sex, race, diet, muscle mass, drugs and methods laboratory analysis.4

Lower values have no clinical relevance.
Presentation

<table>
<thead>
<tr>
<th>Product</th>
<th>Reference</th>
<th>Contents</th>
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<tbody>
<tr>
<td>Creatinine K Vet</td>
<td>1010-300</td>
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<td>Standard 3</td>
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<tr>
<td></td>
<td></td>
<td>Reagent 4</td>
</tr>
</tbody>
</table>

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.