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# CREATININE Kinetic method

Reagent for quantitative determination of Creatinine in human serum and urines

REF 80107 **R1** 1 x 125 mL **R2** 1 x 125 mL **R3** 1 x 10 mL

CE XXXX

IVD

Made In France

I: corresponds to significant modifications

## INTENDED USE

Reagents and aqueous standard for *in vitro* quantitative kinetic determination of creatinine in human serum and urines. Suitable for use with manual procedure on spectrophotometer, or on automated biochemistry analyzer, this device is intended for use as an aid in diagnosis and monitoring of kidney disorders in adult population. For clinical laboratory use by healthcare professionals.

## GENERALITIES (1) (8)

The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). For this test a precisely timed urine collection (usually 24 hours) and a blood specimen are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum or plasma have been made. Among the various approaches suggested, two have found wide recognition: that of Cockroft and Gault and that based on the results of the MDRD (Modification of Diet in Renal Disease) trial. While the first equation was derived from data obtained with the conventional Jaffé method, a newer version of the second is usable for IDMS-traceable creatinine methods. Both are applicable for adults. In children, the Bedside Schwartz formula should be used.

## PRINCIPLE (4) (5)

Colorimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measured kinetically at 490 nm (490-510), without any pre-treatment step. This reaction has been improved (specificity, speed and adaptability) by the development of an initial-rate method.

## REAGENTS

**R1 CRE** Reagent 1

| | |
|---|---|
| Disodium Phosphate | 6.4 mmol/L |
| Sodium hydroxide | 300 mmol/L |

Attention: GSH05, Met Corr.1: H290 - May be corrosive to metals, Skin Corr. 1B: H314 - Causes severe skin burns and eye damage

Classification due to: Sodium Hydroxide > 2.5% - 10%. P280: Wear protective gloves/protective clothing/eye protection/face protection, P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a poison center/doctor.

For more details, refer to Safety Data Sheet (SDS).

**R2 CRE** Reagent 2

| | |
|---|---|
| Sodium dodecyl sulfate | 0.75 mmol/L |
| Picric acid | 4.0 mmol/L |
| pH 4.0 | |

Working reagent (R1+R2) is classified as R1.

**R3 CRE** Standard

Refer to the batch specific value for urines and batch specific value for serum indicated in the label of the vial and in the certificate of analysis.

EUH210: Safety Data Sheet available on request.

According to (EC) 1272/2008 regulation, reagents R2 and R3 are not classified as dangerous.

## MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Automated Analyzer.

## SAFETY CAUTIONS

- Refer to Safety Data Sheet (SDS) available on request or on www.biolabo.fr.
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

## REAGENTS PREPARATION

Working reagent: R1 (1 volume) + R2 (1 volume). Mix gently to homogenize.

## I STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-30°C, and used as described, reagents are stable:

Unopened:
- Until expiry date stated on the label.

Once opened:
- Transfer the requested quantity, well recap and store at 2-30°C.
- Separate reagents are stable at least 6 months.

Once reconstituted, working reagent is stable:
- For 30 days at 2-8°C when free from contamination.

Discard any reagent if cloudy or if reagent blank at 490 nm > 0.300.
Don't use working reagent after expiry date stated on the label.

## SPECIMEN COLLECTION AND HANDLING (2) (7)

This method has been validated on human serum.
The use of other specimen types, as described in the literature, must be validated by the user. For details about possible specimen interferences, see §Limits and §Performances.

Serum: Do not use hemolyzed or icteric serum.

Plasma: Li-heparin and K2-EDTA.

Stability:
- 7 days at 15-25°C
- 7 days at 2-8°C
- 3 months at -15°C to -25°C (freeze once only)

Urines (24h):
Collect without additives. If urine must be collected with a preservative for other analytes, only hydrochloric acid (14 to 47 mmol/L urine, *e.g.* 5 mL 10 % HCl or 5 mL 30 % HCl per liter urine) or boric acid (81 mmol/L, *e.g.* 5 g per liter urine) may be used.

Stability without/ with preservative):
- 2 days/3 days at 15-25°C
- 6 days/8 days at 2-8°C
- 6 months/3 weeks at -15°C to -25°C (freeze once only)

## LIMITS (1) (2) (3) (5) (9) (10-22)

For low values (pediatric specimen and obese patients), ANSM recommends preferably enzymatic method.

Some antibiotics interfere also with the determination of creatinine according to Jaffé method.

Do not use Jaffé method for the testing of creatinine in hemolyzed samples from neonates, infants or adults.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

The presence of ketone bodies can cause artificially high results in serum.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

## CALIBRATION (6)

- REF 95015 Multicalibrator with serum as specimen *(traceable to SRM967b)*
- Or vial R3 (aqueous standard) to be used with:
  - Serum (value traceable to *SRM967b)*
  - Urines (value traceable to *SRM914)*

1 zero-point, 1 intermediate level and 1 high level have been used to determine these values considering ANSM recommendations.

The calibration frequency depends on proper instrument functions and on preservation of the reagents.

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

## I REFERENCE INTERVALS (1)

| Serum (Adult) | mg/dL | [ µmol / L ] |
|---|---|---|
| Male | 0.7 to 1.3 | [ 62-115 ] |
| Female | 0.6 to 1.1 | [ 53-97 ] |

| Urines (Adult) | mg / kg / 24 h | [ µmol / kg / 24 h ] |
|---|---|---|
| Male | 14 to 26 | [ 124-230 ] |
| Female | 11 to 20 | [ 97-177 ] |

Glomerular Filtration Rate (GFR by Creatinine Clearance):

| Adult (20-29 years) | mL per minute/1.73 $m^2$ |
|---|---|
| Male | 94-140 |
| Female | 72-110 |
| Decreases approximately 6.5 mL/min per decade | |

Each laboratory should establish its own normal range for the population that it serves.

## PERFORMANCES

Serum: On KENZA 240TX, at 37°C, 505 nm

Measuring range: between 0.25 (LOQ*) and 15 mg/dL

**LOQ: Limit Of Quantification*

Detection limit: approx. 0.09 mg/dL

Precision:

| *Within-run* N = 20 | *Level 1* | *Level 2* | *Level 3* |
|---|---|---|---|
| Mean (mg/dL) | 0.66 | 1.60 | 5.75 |
| S.D. (mg/dL) | 0.012 | 0.021 | 0.092 |
| C.V. (%) | 1.8 | 1.3 | 1.6 |

| *Between-run* N = 20 | *Level 1* | *Level 2* | *Level 3* |
|---|---|---|---|
| Mean (mg/dL) | 0.66 | 1.64 | 5.81 |
| S.D. (mg/dL) | 0.026 | 0.058 | 0.133 |
| C.V. (%) | 4.0 | 3.5 | 2.3 |

On-board stability: Reagents are stable 8 days

Calibration Frequency: 4 days

Interferences:

| | |
|---|---|
| Turbidity | No interference up to 0.273 OD |
| Total bilirubin | Negative interference from 181 µmol/L |
| Direct bilirubin | Negative interference from 29 µmol/L |
| Ascorbic acid | No interference up to 2500 mg/dL |
| Glucose | No interference up to 1048 mg/dL |
| Hemoglobin | Negative interference from 105 µmol/L |

Other substances may interfere (see §Limits)

Comparison with commercially available reagent on KENZA ONE analyzer with human serums (n=123) between 0.41 and 13.6 mg/dL

y (mg/dL) = 1.1925x - 0.113  R = 0.9879

## REFERENCES


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*(3) Young DS. Effects on Clinical Laboratory Tests – Drugs Disease, Herbs & Natural Products, https://clinfx.wiley.com/ aaccweb/aacc/, accessed in September 2021. Published by AACC Press and John Wiley and Sons, Inc. Clin Chem Lab Med 2007;45(9):1240-1243*

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*(5) D. Labbé et al., Ann. Biol. Clin. (1996), 54, p. 285 – 298*

*(6) SRM: Standard Reference Material ®*

*(7) Guder W, Fonseca-Wollheim W, Ehret W, et al. Die Qualität Diagnostischer Proben, 6. Aufl. Heidelberg: BD Diagnostics, 2009.*

*(8) Schwartz GJ, Muñoz A, Schneider MF, et al. New Equations to Estimate GFR in Children with CKD. J Am Soc Nephrol 2009;20:629-637.*

*(9) Recommandations relatives au dosage de la créatinine (Contrôle de marché 2008-2010) », Agence française de sécurité sanitaire des produits de santé (AFSSAPS)*

*(10-22) More details in Performances detailed report*

*(23) Effect of serum creatinine difference between Jaffe and enzymatic method on kidney disease detection and staging Clinical Kidney Journal, 2023, vol. 16, no. 11, 2147–2155*

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## QUALITY CONTROL

- REF 95010 EXATROL-N
- REF 95011 EXATROL-P
- REF 95012 Urinary controls (to be diluted as sample before test)
- ANSM recommends low, medium and high controls.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat the test with a new vial of reagent.

If control is still out of range, contact BIOLABO Support or your local Agent.

## PROCEDURE

Manual method:

Let stand reagents and specimens at room temperature.

Kinetic test to be performed tube by tube at constant temperature (37°C).

Blank: Replace specimen by demineralized water.

| | |
|---|---|
| Working Reagent (R1+R2) pre-incubated 37°C | 1000 µL |
| Calibrator, Control or Assay | 100 µL |
| Mix well. After 30 seconds read absorbance A1 and exactly 120 sec after read absorbance A2 at 490 nm (490-510). | |

1- Calibrator as indicated in §Calibration

2- Above linearity limit, dilute specimen (1+4) with saline and re-assay considering the dilution factor 5.

3- Urine: pre-dilute (1+19) in demineralized water before assay.

4- Performances with manual procedure should be validated by user.

5- Applications for KENZA analyzers and other applications proposal are available on request.

## CALCULATION (1) (10) (23) (24)

Serum

$$\text{Result} = \frac{\text{(A2 - A1) Assay - (A2 - A1) Blank}}{\text{(A2 - A1) Calibrator - (A2 - A1) Blank}} \times \text{Calibrator Concentration}$$

Note:

To correct from non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, it is generally recommended to apply subtractive compensation between 20 and 30 µmol/L (approximately 0.2 to 0.3 mg/dL), depending on the reagent formulation and the analyzer used. This correction factor must be established by the final user.

Due to the variability of concentration of these pseudo-chromogens, this approach may sometimes lead to aberrant results in children < 3 years and in elderly patients.

Urine: Multiply the above result by dilution factor 20.

Adult GFR (by creatinine clearance estimation):

- Using serum creatinine (Cockcroft and Gault formula)

$$\text{Creatinine Clearance (mg/mL/1.73m}^2\text{)} = \frac{\text{140 – age (years) x 2.12 x weight (Kg) x K}}{\text{Serum Creatinine (µmol/L) x BSA}}$$

- Using 24 h urine and serum creatinine

$$\text{Corrected Creatinine Clearance (mL/min)} = \frac{\text{UCr x V}}{\text{SCr x 1440}}$$

With: K = 1.00 for men or 0.85 for women

BSA = Adult Body Surface Area (Adult 1,73 $m^2$)

UCr = Urine Creatinine in mg/dL or µmol/L

SCr = Serum Creatinine in mg/dL or µmol/L

V = Urine volume excreted in mL/ 24 h (equivalent to 1440 minutes)

**Table of symbols**

| | | | | | |
|---|---|---|---|---|---|
| Manufacturer | Expiry date | In vitro diagnostic | Storage temperature | Demineralized water | Biological risk |
| Product Reference | See Insert | Batch number | Store away from light | Sufficient for | Dilute with |