

REF 10260-4 4 X 29 ML / 10 ML

URINE CREATININE (CREA-U)

Wedges each contain usable volumes of 29 mL of R1 reagent and 10 mL of R2 reagent.

INTENDED USE

The EasyRA CREA-U reagent is intended for the quantitative determination of urine creatinine in human urine, using the MEDICA EasyRA® Chemistry Analyzer in clinical laboratories. Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis and as a calculation basis for measuring other urine analytes.

For *in vitro* diagnostic use only. For professional use only.

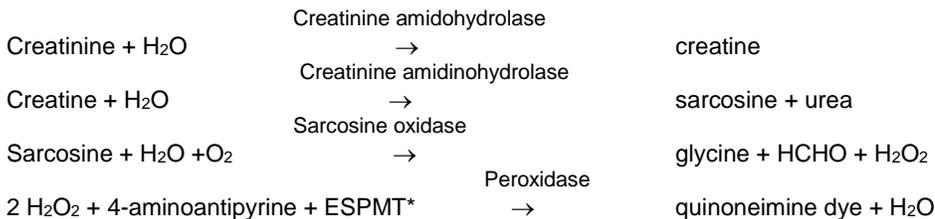
SUMMARY AND EXPLANATION

Clinical Use - Creatinine is the end product of degradation of creatine phosphate, which is used as an energy source for muscular contraction. Creatinine is excreted by the kidney at a constant rate of ~ 2% of body creatine every 24 hours.¹ Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes.² Urine creatinine levels can be used as a screening test to evaluate kidney function, or as part of the creatinine clearance test. The creatinine clearance is an estimate of the glomerular filtration rate, that is, the volume of filtrate made by the kidneys per minute. The urine and serum creatinine levels are measured along with the urine volume in 24 hours. The clearance rate is then calculated. The calculation uses a correction factor for body size. The creatinine clearance appears to decrease with age (each decade corresponds to a decrease of about 6.5 ml/min./1.73 m²).

Specimen Validity Use – A complete urine drug of abuse testing program involves specimen collection, initial screening with an immunoassay, followed by a confirmatory test such as gas or liquid chromatography/mass spectrophotometry (GC/MS or LC/MS).⁷ Pain management and drugs of abuse testing programs consist of protocols to ensure the validity clinical specimens. Drug users may attempt to evade detection by adulterating urine specimens. Adulteration methods include, among others, substitution with or diluting with water or saline to prompt a negative result. Normal Creatinine values in human urine range from 80 – 200 mg/dL and is fairly constant. Urine creatinine values of < 20 mg/dL may indicate adulteration of urine by dilution and values < 2 mg/dL may indicate substitution.⁸

PRINCIPLE OF THE PROCEDURE

This method uses two reagents to perform the enzymatic reaction.



where ESPMT is N-ethyl-N-sufopropyl-m-toluidine

REAGENTS

Creatinine Enzyme Buffer Reagent (R1):

| | |
|---------------------------|-----------|
| Good buffer (pH 7.4) | 25 mmol/L |
| Creatine amidinohydrolase | >25KU/L |
| Sarcosine oxidase | > 7 KU/L |
| Ascorbate oxidase | > 4 KU/L |
| ESPMT | 140 mg/L |

Creatinine Enzyme Color Reagent (R2):

| | |
|---------------------------|------------|
| Good buffer (pH 7.3) | 100 mmol/L |
| Creatinine amidohydrolase | >250 KU/L |
| Peroxidase | > 5 KU/L |
| 4-aminoantipyrine | 600 mg/L |

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent (CLSI GP17-A2).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for risk, hazard and safety information.

3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. The reagent is stable on-board in the refrigerated reagent chamber of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known urine control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clinical Use (24 hour urine) - Use fresh, clear, urine (24 hr) without additives. Centrifuge the urine if it contains particulate material. Creatinine in urine samples is stable for 2 days at room temperature (20 - 25°C), 6 days refrigerated (4-8°C) and 6 months frozen (-20°C).² Frozen Samples should be thawed and mixed thoroughly prior to testing.

Specimen Validity - Use fresh, clear, urine collected in glass or plastic containers without additives. Centrifuge the urine if it contains particulate material. Creatinine in urine samples is stable for 2 days at room temperature (20 - 25°C), 6 days refrigerated (4-8°C) and 6 months frozen (-20°C).² Frozen Samples should be thawed and mixed thoroughly prior to testing.

PROCEDURE

Materials Provided:

Medica CREA-U Reagent Wedge, REF 10203

Additional Materials Required

Medica Creatinine Urine Calibrator, REF 10652
 Commercially Available Urine Control Material
 Medica Precision Test Dye Wedge, REF 10764
 Medica Cleaner (Chem & ISE) Wedge, REF 10660 *or*
 Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use

The reagent is ready to use as supplied. Remove the caps and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (60 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate clean swabs or disposable pipettes for R1 and R2.

Calibration

Medica Creatinine Urine Calibrator, REF 10652, is recommended for the calibration of the assay. The calibration interval (60 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

It is recommended that two levels of controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results

After completion of the assay, the EasyRA Analyzer calculates the creatinine concentration in urine from the ratio of the corrected unknown sample's absorbance to the corrected absorbance of the calibrator multiplied by the calibrator value.

$$\text{CREA-U (mg/dL)} = \frac{[(A_{U_{550}} - A_{U_{700}}) - (A_{RBlk_{550}} - A_{RBlk_{700}})] - [(A_{U_{550}} - A_{U_{700}})_{SBlk} - (A_{RBlk_{550}} - A_{RBlk_{700}})_{SBlk}]}{[(A_{C_{550}} - A_{C_{700}}) - (A_{RBlk_{550}} - A_{RBlk_{700}})] - [(A_{C_{550}} - A_{C_{700}})_{SBlk} - (A_{RBlk_{550}} - A_{RBlk_{700}})_{SBlk}]} \times \text{dF} \times \text{Cal Value}$$

Where A_U and A_C are the absorbance values of the unknown and the calibrator, respectively; A_{RBlk} is absorbance of the reagent blank; $SBlk$ is sample blank; and "Cal Value" is the concentration of creatinine in the calibrator (mg/dL). Since the volume of the reaction is changed with the delayed addition of the R2 reagent, there is a dilution correction factor (dF) included in the calculation.

Expected Values

Clinical Use

The reference range for urine creatinine is based on the concentration in a 24 hr. collection.

Urine creatine (24-hour sample) values may therefore be quite variable and can range from 500 mg/day to 2000 mg/day. The reference range for males is 1000-2000 mg/24 hrs.³ The reference range for females is 600-1500 mg/24 hrs. These values are suggested guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist among instruments, laboratories and local populations.

Specimen Validity

Urine creatinine normal values in human urine range from 80-200 mg/dL. Urine samples with < 20 mg/dL are considered adulterated. Urine samples with < 2 mg/dL are considered adulterated by substitution.⁸

Procedural Limitations (e.g., if sample is above assay range)

The EasyRA Chemistry Analyzer flags any result above 300 mg/dL as Linearity High "LH". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the CREA-U test to 600 mg/dL.

PERFORMANCE CHARACTERISTICS⁴

Reportable Range

The reportable range is 1 to 300 mg/dL. Extended range is 1 to 600 mg/dL when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (CLSI, EP9-A2)

The following table lists the data obtained in a comparison of the Medica CREA-U reagent (y) on the EasyRA Analyzer (y) to the performance of a similar urine creatinine reagent (x) on the Roche COBAS MIRA* Analyzer. Values ranged from 5 to 296 mg/dL. The data shown below are the results for single results obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the Roche COBAS MIRA Analyzer.

| | | | |
|-------------------------|--------|----------------------|---------------------|
| Number of samples | 61 | Range of Samples | 5 to 296 mg/dL |
| Slope | 1.0000 | y Intercept | 0.54 |
| Correlation Coefficient | 0.9849 | Regression Equation: | Y = 1.0000*X + 0.54 |

*Cobas Mira is a registered trademark of Roche Diagnostics, INC., Indianapolis, IN.

Imprecision (CLSI, EP5-A2)

Duplicate measurements of each of four levels of urine QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

| QC Level mg/dL | Within Run SD mg/dL | Within Run CV % |
|-------------------|------------------------|--------------------|
| 70.0 | 1.76 | 2.5 |
| 154.1 | 1.72 | 1.1 |
| 85.2 | 0.74 | 0.9 |
| 194.9 | 1.96 | 1.0 |

Total Imprecision:

| QC Level mg/dL | Total Imprecision SD mg/dL | Total Imprecision CV % |
|-------------------|-------------------------------|---------------------------|
| 70.0 | 1.93 | 2.8 |
| 154.1 | 3.04 | 2.0 |
| 85.2 | 1.53 | 1.8 |
| 194.9 | 2.92 | 1.5 |

Linearity (CLSI, EP6-A)

Linear from 1 to 300 mg/dL.

Interfering Substances (CLSI, EP7-A)

Young gives a list of drugs and other substances that interfere with clinical laboratory tests.^{5,6}

REFERENCES

- 1 Tietz NW. Fundamentals of Clinical Chemistry, PA: WB Saunders Company, 3rd ed., 1987; p 679.
- 2 Ehret W, Heil W, Schmitt Y., Topfer G., Wisser H, Zawta B., et. Al. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99. 1 Rev. 2:22pp.
- 3 Larsen K. Clin Chim Acta 41: 209, 1972.
- 4 Data on file at Medica.

- 5 Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th ed. Washington, DC: AACC Press; 1995.
- 6 Young DS. Effects of Preamalytical Variables on Clinical Laboratory Tests. 2nd ed. Washington, DC. AACC Press;1997.
- 7 *Mandatory Guidelines for Federal Workplace Drug Testing Programs*, National Institute On Drug Abuse. Federal Register Vol. 73. No. 228, 2008;71877.
- 8 *Clinical Drug Testing in Primary Care: Technical Publication Series: TAP 32*. Substance Abuse and Mental Health Administration (SAMHSA), (2012).

EasyRA PARAMETERS (CREA-U)

| | |
|--------------------------------|--------------------------------------|
| Primary Wavelength (nm) | 550 |
| Secondary Wavelength (nm) | 700 |
| Reaction Type | Endpoint, sample blank corrected (2) |
| Reagent Direction | Increase |
| Reagent Blank | Yes (with each calibration) |
| Sample Blank | Yes |
| Blank high Abs. limit | 0.10 |
| Reaction Time | 10 min |
| Calibration interval (maximum) | 60 days |
| Reagent on-board stability | 60 days |

Urine

| | |
|--------------------------|---------------------------------|
| Urine volume (µl) | 2.0 |
| Diluent 1 volume (µl) | 10 |
| Diluent 2 volume (µl) | 10 |
| Reagent volume R1 (µl) | 180 |
| Reagent volume R2 (µl) | 60 |
| Decimal Places (default) | 0 |
| Units (default values) | mg/dL |
| Dilution Factor | 1:1 (to extend measuring range) |
| Linearity | 1 to 300 mg/dL |