MEDICA

IVD CE

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
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 m^2).

PRINCIPLE OF THE PROCEDURE
This method uses two reagents to perform the enzymatic reaction.

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine amidohydrolase}} \text{creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatinine amidinohydrolase}} \text{sarcosine} + \text{urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosine oxidase}} \text{glycine} + HCHO + H_2O_2$$

$$2\,H_2O_2 + \text{4-aminoantipyrine} + \text{ESPMT*} \xrightarrow{\text{Peroxidase}} \text{quinoneimine dye} + H_2O$$

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 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 light-sensitive. Protect reagent wedges from light during storage prior to placing on the analyzer.** Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY
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]

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.

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

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
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.

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 CHARACTERISTICS[4]

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

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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 Preanalytical Variables on Clinical Laboratory Tests. 2nd ed. Washington, DC. AACC Press;1997.

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

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EC REP

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