MEDICA

IVD CE

REF 10203-4 4 x 29 mL / 10 mL

CREATININE (CREA)

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

INTENDED USE

The EasyRA® CREA reagent is intended for the quantitative determination of creatinine in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA "EasyRA® Clinical Chemistry Analyzer." 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]

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 the 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. 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

Clear unhemolyzed serum and plasma should be used. Lithium heparin coated tubes may be used for plasma collection. Centrifuge and remove the serum as soon as possible after collection. Serum creatinine is stable for 3 days at 2 – 8°C and at -20°C for longer periods. Use plasma samples within 24 hours of collection.

PROCEDURE

Materials Provided:
Medica CREA Reagent Wedge, REF 10203

Additional materials required
Medica EasyCal Chemistry, REF 10651
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. When used this way, the reagent is stable on-board in the refrigerated area of the EasyRA Analyzer for the number of days programmed on the RFID Chip on the reagent wedge (60 days maximum).

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

Calibration
Medica EasyCal Chemistry (REF 10651) is recommended for the calibration of the assay. The calibration interval (20 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 human serum based 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 from the ratio of the corrected unknown sample's absorbance to the corrected absorbance of the calibrator multiplied by the calibrator value.

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

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 CREA in serum plasma is as follows:[2]

Normal: 0.5 – 1.2 mg/dL

These values are 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)
Avoid using hemolyzed serum samples.

The EasyRA Analyzer flags any result above 15 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 test to 30 mg/dL.

PERFORMANCE CHARACTERISTICS[3]

Reportable Range
The reportable range is 0.20 to 15.00 mg/dL. Extended range is 0.20 to 30.00 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 Reagent for CREA (y) on the EasyRA Analyzer to the performance of prior Medica Reagent for CREA (x) on the EasyRA Analyzer. The data for serum shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate values obtained for the prior CREA reagent on the EasyRA Analyzer.

Number of samples	70	Range of Samples	0.22 to 14.60 mg/dL
Slope	1.0070	y Intercept	-0.0594
Correlation Coefficient	0.9999	Regression Equation	Y = 1.0070*X – 0.0594

The following table lists the data obtained in a comparison of matched serum (x) and Li-Heparinized plasma (y) samples using the Medica Reagent for CREA on the EasyRA Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	22	Range of Samples	0.23 to 14.07 mg/dL
Slope	1.0063	y Intercept	-0.0410
Correlation Coefficient	0.9999	Regression Equation	Y = 1.0063*X – 0.0410

The following table lists the data obtained in a comparison of the prior Medica Reagent for CREA (y) on the EasyRA Analyzer to the performance of a similar CREA reagent (x) on the Roche COBAS MIRA* Analyzer. The data shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the COBAS MIRA Analyzer.

Number of Samples	62	Range of Samples	0.47 to 14.37 mg/dL
Slope	1.0449	y Intercept	-0.0819
Correlation Coefficient	0.9994	Regression Equation:	Y = 1.0449*X – 0.0819

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

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels of 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 %
6.73	0.04	0.66
1.24	0.01	1.07
0.72	0.01	1.25

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
6.73	0.09	1.3
1.24	0.02	1.5
0.72	0.02	2.5

Linearity (CLSI, EP6-A)
Linear from 0.20 to 15.0 mg/dL, based on the linear regression Y = 1.0387*X – 0.1646.

Limit of Blank (LOB):	0.02 mg/dL	(CLSI, EP17-A)
Limit of Detection (LOD):	0.04 mg/dL	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% interference was classified as “no significant interference.”
There is significant interference at hemoglobin levels above 300 mg/dL Avoid using hemolyzed serum or plasma samples.
No significant interference was found in levels up to 30 mg/dL of bilirubin.
No significant interference was found in levels up to 30 mg/dL of ascorbic acid.
No significant interference was found in levels up to 5 mg/dL of creatine.
No significant interference was found in levels up to 890 mg/dL of triglycerides (using Intralipid*).

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[4]

REFERENCES

1 Tietz NW. Fundamentals of Clinical Chemistry, PA: WB Saunders Company, 3rd ed., 1987; p 679.
2 Tietz NW. Fundamentals of Clinical Chemistry, PA: WB Saunders Company 6th ed. 2008 p. 844.
3 Data on file at Medica.
4 Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th ed. Washington, DC: AACC Press; 1995.

EasyRA Assay Parameters (CREA)

Primary Wavelength (nm)	550
Secondary Wavelength	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	8 min
Calibration interval (maximum)	20 days
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	5.0
Diluent 1 Volume (µl)	10
Diluent 2 Volume (µl)	10
Reagent Volume R1 (µl)	180
Reagent Volume R2 (µl)	60
Decimal Places (default)	2
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend measuring range)
Linearity	0.20 to 15 mg/dL

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