UMA CO., LTD.
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Matsudo, Chiba, Japan

MEASURE CRE

Reagent for determination of Creatinine
SOD/POD Method

2 ~ 8 °C

IVD *In vitro* Diagnostics

DO NOT freeze

18 months/block from light

QUALITY MANAGEMENT SYSTEM (BY TUV)
= ISO 13485:2016 =

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the Creatinine (CRE) concentration in serum, plasma or urine.

2. GENERAL INSTRUCTION

a. For in vitro diagnostics use only.
b. Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.
c. For guaranteed results, usage of this product must comply with the instruction in this manual.
d. If you use automatic analyzers, follow their instructions carefully.

SUMMARY

Creatinine is a break-down product of creatine phosphate in muscle tissue. It is usually produced at a fairly constant rate. Creatinine is cleared by the kidneys with minimal tubular reabsorption. Creatinine accumulates in the blood when GFR decreases in the setting of renal dysfunction. As a result, serum creatinine levels are commonly used as a surrogate for GFR and renal function. Since renal dysfunction is a negative prognostic factor in patients with heart failure, elevations in serum creatinine levels are associated with poor outcomes in heart failure patients. These studies highlight the fact that elevated creatinine level is a strong predictor of mortality in patients with heart failure and can serve as a fast and inexpensive biomarker to identify patients at high risk for adverse outcomes.

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water
- Micropipet and other basic laboratory equipment.
- Calibrators and Controls (separatedly sold)

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: Creatinase, Sarcosine oxidase, (SROD)N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylanilie sodium salt (TOOS)

Reagent R-1 is ready for use

- Reagent R-2: Creatininase (CRN), Peroxidase (POD), 4-Aminoantipyrine (4-AA)

Reagent R-2 is ready for use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.
- Applicable to various automated analyzers.
- Calibrator MEASURE Multi Calibrator (separately sold): Put 5 mL of purified water to the vials of Calibrator (MEASURE Multi Calibrator), leave at room temperature for 45 minutes and sometimes gently invert the vial before use. After reconstituting, Calibrator can be used without dilution.
- Controls MEASURE Human Lyo L-1 and MEASURE Human Lyo L-2 (separately sold): Put 5 mL of purified water to the vials of controls (Lyo L-1 and Lyo L-2); leave at room temperature for 45 minutes and sometimes gently invert the vial before use. After reconstituting, controls can be used without dilution.

5. SAMPLE PREPARATION & STORAGE

- Serum: Wait until the sample is completely coagulated. Take the supernatant to use as specimens.
- Plasma: Treat blood sample by anticoagulant (Li-heparin and K2-EDTA plasma); leave it to stand for 3 hours or centrifuge at 2000 rpm for 2 minutes; take the plasma layer (supernatant) and use as specimen.
- Urine: Collect urine without using additives. If urine must be collected with a preservative for other analysis, 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
- Analyze samples soon after collection.
- Stability in serum/plasma:
  - 7 days at 15-25°C
  - 7 days at 2-8°C
  - 3 months at (-15)-(-25)°C
- Stability in urine (without preservative):
  - 2 days at 15-25°C
  - 6 days at 2-8°C

Revision 09/2022

- 6 months at (-15)-(-25)°C

- Stability in urine (with preservative):
  - 3 days at 15-25°C
  - 8 days at 2-8°C
  - 3 weeks at (-15)-(-25)°C

6. MEASUREMENT PRINCIPLE

In the first reaction, endogenous Creatinine and Sarcosine in patient samples are eliminated. In the second reaction, creatininase is used to convert Creatinine in the samples to creatine. Further, through the action of creatinase and Sarcosine Oxidase, hydrogen peroxide is generated. This hydrogen peroxide causes oxidative condensation between 4-aminoanitpyrine and TOOS under existence of Peroxidase and generate red-violet colored quinone pigment. Concentration of Creatinine in the blood is determined by measuring its absorbance.

1st Reaction

Creatine (endogenous) + H_2O $\xrightarrow{Creatinase}$ Sarcosine + Urea

Sarcosine (endogenous) + O_2 + H_2O $\xrightarrow{SOD}$ Glycine + HCHO + H_2O_2

2nd Reaction

Creatinine + H_2O $\xrightarrow{Creatininase}$ Creatine

Creatine + H_2O $\xrightarrow{Creatinase}$ Sarcosine + Urea

Sarcosine + O_2 + H_2O $\xrightarrow{SOD}$ Glycine + HCHO + H_2O_2

$2H_2O_2$ + 4-AA + TOOS + H_3O^+ $\xrightarrow{POD}$ Red-violet quinone pigment + $5H_2O$

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.

Sample	+	Reagent 1	$\xrightarrow[265s]{37^oC}$	Reagent 2
3.8µL		180µL		60µL

$\xrightarrow[18s]{37^oC}$ Meas. Abs I $\xrightarrow[317s]{37^oC}$ Meas. Abs II

(546/800nm) (546/800nm)

⟶ ∆Abs at 546/800nm ⟶ Concentration

Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact HUMA MEDICAL CO., LTD. for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ∆Abs of specimen & standards vs blank
- Plot a calibration curve CRE (U/L) = f(∆Abs)
- Calculate CRE concentration in specimen using the curve (doing same procedure for Controls)

Unit conversion

mg/dLx 88.4 = µmol/L

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- With Serum & Plasma

0.1 ~ 100 mg/dL (8.84 ~ 8840 µmol/L)

- If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

b. Lower Detection Limit: 0.1 mg/dL

The lowest detectable level represents the lowest measurable level of CRE that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analysis free sample.

Values below the lower detection limit (< 0.1 mg/dL) will not be detected by this method.

c. Performance

- Sensitivity: Using purified water, absorbance change is 0.001 ~ 0.050; using solution of Creatinine 5 mg/dL, absorbance change is 0.040 ~ 0.200.
- Specificity: The accuracy is within ±10.0%.
- Reproducibility: CV value < 10.0%.

d. Correlation Test (n = 50)

Serum:	Regression equation	y = 0.9872x + 0.012
	Correlation coefficient	r = 0.9747
Plasma	Regression equation	y = 1.0138x - 0.0149
	Correlation coefficient	r = 0.9203
Urine	Regression equation	y = 1.0011x - 0.0088
	Correlation coefficient	r = 0.9999

Reference Materials for Calibration

- JCCRM 521

10. EXPECTED VALUES

- Serum	Male:	0.6 ~ 1.1 mg/dl	(53 ~ 106µmol/L)
	Female:	0.4 ~ 0.8 mg/dl	(35 ~ 71µmol/L)
- Urine		700 ~ 1800 mg/24h	

Reference range should be established at each facility and judgement should base on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated bilirubin concentration up to 20 mg/dL
- Hemolysis: No significant interference of hemoglobin concentration up to 500 mg/dL
- Lipemia (Intralipid): No significant interference triglycerides concentration up to 3000 FTU
- Ascorbic Acid: No significant interference of ascorbic acid concentration up to 50 mg/dL
- Anticoagulant: No significant interference with Heparin, EDTA, NaF
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors.

12. HANDLING, USAGE & DISPOSAL

Handling

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.
2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.
3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.
2. Do not use the container and auxiliaries included in this kit for other purposes.
3. Do not mix reagents of different lot for use.
4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

・Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

・Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ **For Hitachi Model**

Calculation Method		2-point (fix)
Temperature		37°C
Volume (µL)	Specimen	3.8
	R1	180
	R2	60
Wavelength (nm)	Main	546
	Sub-	800
Measurement (cycle)	Point 1	10
	Point 2	16
	Point 3	34
Calibration type		Linear
Unit		mg/dL

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.
- Prepare the calibration curve on the day of determination.

15. PACKING AND KIT CONFIGURATION

Product Code	Product Name	Packages
11C013A	Measure CRE	1x60mL; 1x20mL
11C003A	Measure CRE	5x60mL; 5x20mL
11C013	Measure CRE	1x90mL; 1x30mL
11C003	Measure CRE	3x90mL; 3x30mL

Constituent reagents are available in other configurations. For further details please contact HUMA MEDICAL CO., LTD.

16. REFERENCES

1. Y. Xue, ... Navaid Iqbal, in Reference Module in Biomedical Sciences, 2014
2. In house data, UMA Diagnostics.

17. MANUFACTURER

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