Creatinine in Plasma/Serum/Urine and Body Fluid

Purpose

This procedure provides instructions for performing the *in vitro* diagnostic test for CREATININE IN PLASMA/SERUM/URINE/BODY FLUID. The ECREA method is an *in vitro* diagnostic test for the quantitative measurement of creatinine in human serum, plasma and urine on the Dimension Vista® System. Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for other urine analytes.

Policy Statements

This procedure applies to all personnel who run the Siemens Dimension Vista 500 and the Dimension RxL MAX.

Principle

Both creatinine methods measure creatinine enzymatically. Enzymatic creatinine methods are reported to be less susceptible to interference than Jaffe methods from non-creatinine substances.

PRIMARY METHOD: (ECRE)

In a coupled enzyme reaction, creatininase hydrolyzes creatinine to creatine, which is hydrolyzed by creatinase to sarcosine. Sarcosine oxidase hydrolyzes sarcosine to glycine, formaldehyde, and peroxide. The peroxide and a chromogen in the presence of peroxidase form a colored end product that is proportional to the amount of creatinine in the sample. The colored reaction product is measured at 540 and 700 nm.

SECONDARY METHOD: (EZCR) Test results of the Dimension® EZCR method correlate with those obtained by an IDMS-traceable method. In a 3-[[tris(hydroxymethyl)methyl]amino]propanesulfonic acid (TAPS) buffered coupled enzyme reaction, creatininase hydrolyzes creatinine to creatine, which is hydrolyzed by creatinase to sarcosine. Sarcosine oxidase hydrolyzes sarcosine to glycine, formaldehyde, and peroxide. The peroxide and a chromogen in the presence of peroxidase form a colored end product that is proportional to the amount of creatinine in the sample and is measured using a bichromatic (540, 700 nm) end point technique.

Clinical Significance

Creatine is involved in energy storage in skeletal muscle and other tissues and is synthesized in the liver from amino acids and transported in the blood to muscle. There the enzyme CPK catalyzes the reaction of creatine with ATP to form phosphocreatine. Creatinine is a catabolic end product. Creatinine is excreted from the body via the urine and is formed at a nearly constant rate proportional to the body muscle mass.

The amount of creatinine excreted in the urine is primarily a function of glomerular filtration. Measurement of the amount of creatinine produced in urine during a specified period of time and the plasma creatinine level allows one to calculate a value called the creatinine clearance. This is measured in milliliters of plasma per minute, and represents the volume of plasma that must be filtered by the kidney to account for the amount of creatinine found in the urine. The value obtained for creatinine clearance correlates fairly well with more exact measures of glomerular filtration rate (GFR).

Serum and plasma creatinine concentrations, freely filtered by the glomerulus, are inversely related to glomerular filtration rate (GFR). Therefore, both blood and urine creatinine measurements are used to assess kidney function and in the diagnosis and treatment of renal diseases. Creatinine is also useful in monitoring effectiveness of renal dialysis. Calculated creatinine clearance values may be used to establish effective therapeutic dosing levels of pharmaceuticals.

The Urine Calcium/ Creatinine ratio is useful in determining the presence of idiopathic hypercalciuria in patients with unexplained hematuria, especially when the family history is positive for renal calculi.

Analyzer

PRIMARY METHOD: Siemens Dimension Vista 500
SECONDARY (BACKUP) METHOD: Siemens Dimension RxL MAX
Backup Method for URINES: Vista on other campus

Test Codes

Sunquest test codes for Creatinine	
CREA	CREA
UCRE	UCRE
FCRE	Fluid Creatinine
CR24	24 hour urine Creatinine
CRCL	Creatinine Clearance
CCRA	Calcium Creatinine Ratio on urine
UMAR	Urine Microalbumin Creatinine Ratio
UPCR	Protein Creatinine Ratio on urine

Sample

1. Venipuncture should occur prior to administration of N-Acetylcysteine (NAC) or Metamizole due to the potential for falsely depressed result[11]. NAC is the accepted antidote for acetaminophen toxicity. Metamizole is an anti-pyretic banned in most countries.
2. Plasma (lithium heparin) preferred or serum. Refer to specimen collection procedures for collection of plasma and serum samples
3. Urine: Timed (24 hour) or Random collection no preservatives. Specimens previously preserved with 6N HCl or Boric Acid are acceptable.
4. Body fluid

Minimum volume: 0.2 mL (actual test volume: 2.7 µL Vista, 6 µL RxL MAX)

Stability:

- Serum/plasma: 2-8 °C / 48 hours, -20 °C / 1 month
- Urines 24 hour or random: 2 – 8 ºC / 4 days, < -20 °C / 1 month

Rejection criteria: Unlabelled specimens.

Preparation

1. Complete clot formation should take place before centrifugation. Serum or plasma should be physically separated from cells with a maximum limit of 2 hours from the time of collection. Specimens should be free of particulate matter. Grossly hemolyzed samples should not be used with the EZCR or ECREA method.
2. Whole blood specimens should be centrifuged according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
3. Collect random and timed urine specimens using recommended procedures for collection, transportation, and preservation of urine specimens. See Specimen Collection Manual. Urine creatinine measurements require no special patient preparation for specimen collection
4. Timed urine collections are measured for total volume, and the collection date and time recorded for the start and end of the collection.
    a. Centrifuge an aliquot to obtain a clear supernatant for testing on the analyzer.
    b. Enter collection information into Sunquest by ordering the test PV on the same accession number.
    c. The patient's height and weight must be entered at the point of order, or by the lab staff for the creatinine clearance calculation. In addition, a serum/plasma creatinine result must be available in Sunquest for the LIS to calculate CRCL.
        i. To convert pounds to kilograms, divide the patient's weight in pounds by 2.2.
        ii. To convert inches to centimeters, multiply height in inches by 2.54.
5. Body fluid specimens must be liquid and must be centrifuged until a clear supernatant similar to serum or plasma is obtained

Reagents

PRIMARY METHOD: (ECRE)

Product Description	*Product Code*	*Stability*
ECREA Flex Reagent Cartridge (Vista) Reagents are liquid and ready-to-use	K1270A	**Store at:** 2 – 8 °C **Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 30 days. **Open well stability:** 3 days for wells 1 - 12.
ECREA CAL, ready for use Traceable to NIST SRM 914 Working calibrators verified traceable to ID-GC/MS	KC270	**Store at:** 2 - 8 °C. **Unopened:** Refer to carton for expiration date. **On-board:** Once the vial stopper is punctured, assigned values are stable for 30 days when stored on board the Dimension Vista® System

SECONDARY METHOD:

Product Description	*Product Code*	*Stability*
EZCR Flex® Reagent Cartridge (RXL) Reagents are liquid and ready-to-use	DF270B	**Store at:** 2 – 8 °C **Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 30 days. **Open well stability:** 5 days for wells 1 - 6
Dimension® CHEM I Calibrator See product insert for reagent preparation instructions.	DC18B	Store at 2 – 8°C Assigned values are stable for 24 hours after reconstitution, stoppered and stored at 2 – 8°C

Safety

Precautions:

ECREA CAL

Irritant. Contains a mixture of 5-chloro-2-methyl-2H-isothiazol -3-one and 2-methyl-2H-isothiazol-3-one (3:1).

- May cause sensitization by skin contact.
- Avoid contact with skin.
- Wear suitable gloves.
- Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics or on Children's MSDS On-line.

For *in vitro* diagnostic use

Calibration

PRIMARY METHOD:

Assay Range (AMR):	Serum/plasma 0.14 – 20.0 mg/dL Urine 2.80 – 400 mg/dL
Reference Material:	ECREA CAL, Cat. No. KC270, NIST SRM 914
Calibration Levels:	3 levels, n = 5
Calibration Scheme:	Level 1 (System water): 0.0 mg/dL Level 2 (Calibrator A): 0.95 mg/dL Level 3 (Calibrator B): 20.0 mg/dL
Calibration Frequency:	• Every 90 days for any one lot • For each new lot of Flex® reagent cartridges • After major maintenance or service, if indicated by quality control results • As indicated in laboratory quality control procedures • When required by government regulations

Analytical Measuring Range (AMR)

- Cal Verification and AMR verification are performed at least once every six (6) months.
- Touch Advanced → Calibrations → Calibrations by Lot, select method ECREA and "Order a Linearity Study"
- See iGuide "Calibration by Lot" for more information.

SECONDARY METHOD:

Assay Range (AMR):	0.03 – 20.00 mg/dL
Reference Material:	Dimension® CHEM I Calibrator Cat. No. DC18B
Calibration Levels:	3 levels in triplicate
Calibration Scheme:	Level 1: 0.00 mg/dL Level 2: 11.00 mg/dL Level 3: 22.00 mg/dL
Calibration Frequency:	• Every 90 days for any one lot • For each new lot of Flex® reagent cartridges • After major maintenance or service, if indicated by quality control results • As indicated in laboratory quality control procedures • When required by government regulations

Analytical Measuring Range (AMR)

Verification of AMR is accomplished with each calibration using at least three calibrators that span the reportable range.

Quality Control (Plasma/Serum)

PRIMARY METHOD: BioRad Mulitqual® 1 & 3 Control Levels, contained in Vista vials.

Frequency: Two levels each day of use

Stability: Refer to the current lot product insert

Sunquest Control names: C-MQ1, C-MQ3

SECONDARY METHOD: Biorad Liquichek™ Unassayed Chemistry Control (Human) Levels 1 & 2

Frequency: Two levels each day of use

Stability: Refer to the current lot product insert

Sunquest Control names: C-X1, C-X2

Quality Control (Urine)

PRIMARY METHOD: (ONLY) Liquichek Urine Chemistry 1 & 2 Control Levels, contained in Vista vials.

Frequency: Two levels each day of use

Stability: Refer to the current lot product insert,

Sunquest Control names: C-UR1, C-UR2

Quality Control Acceptable Ranges

Acceptable ranges: Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.

If a control value is outside the confidence interval, the determination must be repeated. If the repeated determination confirms the deviation, a new reference curve should be established. Do not release patient results until the cause of deviation has been identified and corrected. When a new lot of control is received, validate the manufacturer's insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range.

Calculations

CCRA = Urine Calcium/Creatinine ratio = $\frac{\text{Ca (mg/dL)}}{\text{Creat (mg/dL)}}$

CR24 = mg Creatinine/24 hrs = $\frac{\text{Creat mg/dL x total volume in mL}}{100}$

CRCL = Creatinine Clearance = UV/P*1.73/A where:

U = urine creatinine
P = plasma creatinine
V = volume/time in minutes
A = body mass
NOTE: calculate body mass with the equation $S = M^{0.425} \times H^{0.725} \times 71.84$ where:
S = body surface in cm^2
M = mass in Kg
H = height in cm

To convert pounds to kilograms, divide the patient's weight in pounds by 2.2.
To convert inches to centimeters, multiply height in inches by 2.54.

UPCR = Urine Protein (mg/dL)/Urine Creatinine (mg/dL) = urine protein creatinine ratio

UMAR = Urine Microalbumin (mg/L)/ Urine Creatinine (mg/dL) = Urine Microalbumin/ Creatinine ratio (mg/g)

Interferences

PRIMARY METHOD:

Hemolysis, Icterus & Lipemia (HIL) Index Values:

H	I	L
7	6	8

HIL:

- Hemoglobin at 600 mg/dLdecreases ECREA results by 10% at 1.02 mg/dL ECREA.
- Bilirubin (unconjugated) at 40 mg/dL decreases ECREA results by 19% at 1.01 mg/dL
- Bilirubin (conjugated) at 40 mg/dL decreases ECREA results by 16% at 1.01 mg/dL
- Lipemia at 3000 mg/dL increases ECREA by 25% at 1.01 mg/dL.

Blood samples from patients with Waldenstrom's Macroglobulinemia contain monoclonal IgM that can produce falsely elevated results.

Refer to the Siemens IFU for a list of non-interfering substances.

SECONDARY METHOD:

Hemoglobin at 400 mg/dL increases EZCR results by 15% at a creatinine concentration of 1.00 mg/dL

No interference was found for:

- Commonly used drugs .
- Bilirubin (conjugated) up to 20 mg/dL
- Bilirubin (unconjugated) up to 23 mg/dL
- Lipemia up to 3000 mg/dL

Reference Ranges Serum/Plasma

Expected Values
Serum/plasma: Derived from previous ranges and method correlation

Males:	Reference range	Females:	Reference range
0 – 7 days	0.58 – 1.09 mg/dL	0 – 7 days	0.58 – 1.09 mg/dL
8 days – 1 mo	0.18 – 0.68 mg/dL	8 days – 1 mo	0.18 – 0.68 mg/dL
0-3 years:	0.18 - 0.48 mg/ dL	0-2 year:	0.18 - 0.48 mg/ dL
3-5 years:	0.18 - 0.58 mg/ dL	2-5 years	0.18 - 0.58 mg/ dL
5-6 years:	0.28 - 0.58 mg/ dL	5-6 years	0.28 - 0.58 mg/ dL
6-8 years:	0.28 - 0.68 mg/ dL	6-9 years	0.28 - 0.68 mg/ dL
8-10 years:	0.28 - 0.78 mg/ dL	9-12 years	0.28 - 0.78 mg/ dL
10-12 years:	0.38 - 0.89 mg/ dL	12-15 years	0.38 - 0.89 mg/ dL
12-14 years:	0.38 - 0.99 mg/ dL	15-18 years	0.38 - 0.99mg/ dL
14-16 years:	0.48 – 1.09 mg/ dL	>18 years	0.48 - 0.99 mg/ dL
16-18 years:	0.58 – 1.19 mg/ dL		
>18 years:	0.58 – 1.29 mg/ dL		

Reference Ranges Urine

The reference values are for a 24-hour collection. Random specimens and timed specimens collected for other than a 24-hour time period have no reference values established.

Age	Concentration mg/collection
3 - 8 years	110 - 680 mg/collection
9 - 12 years	170 - 1410 mg/collection
13 - 17 years	290 - 1870 mg/collection
>17 years	630 - 2500 mg/collection

Random Urine

Males: 40 – 278 mg/dL
Females: 29 – 226 mg/dL

Reference Ranges (cont)

Creatinine Clearance:
70 – 130

Calcium/Creatinine Ratio
Up to age 16 < 0.20
Adult < 0.11

Critical Values

None specified.

Limitations

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension RXL or Vista Operator's Guide for troubleshooting.

Dilutions

Above 20.0 mg/dL on **plasma/serum** samples:

ECREA on Serum/Plasma (PRIMARY METHOD)	
Maximum Dilution	1:4
Surplus Rack	Samples with results >20.0 mg/dL are automatically repeated on a higher dilution.
Limited Rack	Samples with results >20.0 mg/dL should be repeated as an Add-On Test with a Special Dilution of 1:4
Manual Dilution	Do not manually dilute.

EZCR on Serum/Plasma (SECONDARY METHOD)	
Maximum Dilution	1:3
Autodilution	Samples with results >20.0 mg/dL are automatically repeated on a 1:3 dilution when the Autodilute feature is enabled
Manual Dilution	Do not manually dilute.

Above 200.0 mg/dL on **Urine** samples:

ECREA Urine (PRIMARY METHOD)	
Predilution (Urine)	1:20
Maximum Dilution	1:40
Surplus Rack	Samples with results >400.0 mg/dL are automatically repeated on a higher dilution (1:40)
Limited Rack	Samples with results >400.0 mg/dL should be repeated as an Add-On Test with a Special Dilution of 1:40
Manual Dilution	Do not manually dilute.

Urine testing is available on the **PRIMARY METHOD** only

Result Reporting

PRIMARY METHOD:

- Results between 0.14 – 20.0 mg/dL without error messages are released
- Serum/Plasma results below 0.14 mg/dL: report as < 0.14 mg/dL
- Results >20.0 mg/dL without error messages are reported following a maximum dilution of 1:4
- Serum/plasma results with assay range appended following the maximum auto-dilution are reported as >80 mg/dL
- Append the comment "HP" (Hemolysis present, may affect results) to hemolyzed samples. Refer to CH5.101 HIL on Dimension Vista 500
- Urine results with below assay range message are reported as <2.8 mg/dL
- Urine results with "assay range" appended following the maximum dilution of 1:40 are reported as >800 mg/dL
- Urine results without error messages: The Laboratory Information System calculates urine creatinine on timed collections, as well as Creatinine Clearance and the Calcium/Creatinine ratio when all necessary information is present. Report the numerical value.

SECONDARY METHOD:

Results between 0.03 – 20.0 mg/dL without error messages are released

- Serum/Plasma results below 0.03 mg/dL: report as < 0.03 mg/dL.
- Results >20.0 mg/dL without error messages are reported following a maximum dilution of 1:3
- Serum/Plasma results with "assay range" appended following a maximum dilution of 1:3 are reported as >60 mg/dL
- Append the comment "HP" (Hemolysis present, may affect results) to hemolyzed samples

Specimen Storage

Promptly stopper tested specimen and store upright in numbered specimen rack by accession number. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer.

References

1. ECREA Flex® Reagent Cartridge insert sheet, Siemens Healthcare Diagnostics Inc., Newark, DE 19714, PN 781270.001 - US, Issue Date 08/20/2013 Rev. C
2. EZCR Flex® Reagent Cartridge insert sheet (RXL/MAX), Siemens Healthcare Diagnostics Inc., Newark, DE, PN 717270.003 - US, Issue Date 2014-11-21 Rev. E
3. ECREA CAL product insert, Dimension Vista ® System, Siemens Healthcare Diagnostics Inc., Newark, DE 19714, PN 751270.001-US, rev D, 03/2012
4. Chemistry 1 Calibrator package insert, Siemens Healthcare Diagnostics Inc., Newark, DE 19714, PN 792018.002-US, Rev E, Issue date 06/2013
5. Clinical Significance, Dade Behring Inc., Glasgow Business Community, Mailbox 531, P.O. Box 6101, Newark, Delaware 19714
6. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc, Hudson, OH, 5th Edition, 2001
7. Pediatric Reference Intervals, Sixth Edition, AACC Press, Washington DC, 2007
8. Biorad Liquichek™ Unassayed Chemistry Control (Human) product insert, Bio-Rad Laboratories, Irvine, CA
9. BioRad Mulitqual® 1 & 3 Control Levels product insert, Bio-Rad Laboratories, Irvine, CA
10. Liquichek Urine Chemistry 1 & 2 Control Levels Product insert, Bio-Rad Laboratories, Irvine, CA 92618
11. Medical Device Correction Notice DC-16-02.A.US – March 17, 2016

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	D. Reidel/L.Lichty	08/2001	Initial Version
2.	L. Lichty	6/5/2005	
3.	L. Lichty	01/01/2007	
4.	L. Lichty	April 1, 2011	Updated package insert information. New format, renumbered from CH 3.20
5.	L. Lichty	February 4, 2013	IDMS traceable reagent validated
6.	L. Lichty	May 6, 2013	Amended urine diluent
7.	L. Lichty	12/17/2013	Siemens CLSI ECREA procedure for Vista, issue date 4/4/2012
8.	L. Marsh/L. Lichty	January 6, 2014	Updated for use on Vista 500
9.	L. Lichty	March 28, 2016	NAC/Metamizole warning