

CREATININE

CR 0500 CH

4 x 125 ml

SUMMARY OF TEST

Between 1 and 2% of muscle creatine is converted to creatinine daily. Because the amount of endogenous creatinine produced is proportional to muscle mass, the production varies with age and sex; nonobese men excrete about 1.5 g/d, women 1.2 g/d. Daily excretion of creatinine can be 10 to 30% greater as a result of dietary intake of creatine and creatinine in meats. Because creatinine is endogenously produced and released into body fluids at a constant rate and its plasma levels maintained within narrow limits, its clearance may be measured as an indicator of glomerular filtration rate (GFR). However, a small quantity of creatinine is reabsorbed by the tubules and a small quantity of creatinine appearing in the urine (7-10%) is due to tubular secretion. As a result, creatinine clearance (if creatinine is measured with an accurate method) is approximately 7% greater than inulin clearance. Some methods for creatinine used in clinical laboratories are nonspecific, however, and thus this difference is often smaller. The creatinine clearance is performed by obtaining a 4-, 12-, or 24-h urine specimen and also a blood specimen sometime within the period of urine collection. The volume of the urine is measured, urine flow rate is calculated (ml/min), and the assay for creatinine is performed on plasma and urine to obtain the concentration in milligrams per deciliter or per milliliter. Two factors influence measurement of creatinine clearance and thus its interpretation. First, the most common methods for measuring creatinine use the nonspecific alkaline picrate reaction, and thus noncreatinine chromogens in plasma increase the apparent plasma concentration by as much as 30% if serum values are less than 1.0 mg/dl and by approximately 10% if values exceed 1.0 mg/dl. The per cent increase is progressively less with higher creatinine concentrations. This overestimation of plasma creatinine concentration results in underestimation of creatinine clearance and partially offsets the apparent high clearance of creatinine that is due to tubular secretion. As a result, the endogenous creatinine clearance agrees closely with the inulin clearance throughout a substantial range of clearances. However, if accurate methods are used for assay of plasma creatinine, the GFR estimated by creatinine clearance may not correlate with the GFR estimated by inulin clearance. Secondly, GFR measured by creatinine clearance and GFR measured by inulin clearance in the same patient progressively diverge as renal failure progresses and plasma creatinine level rises. The greater apparent GFR found by creatinine clearance may be due to an increase in tubular secretory activity for creatinine when plasma levels increase much above normal and to the relatively smaller contribution of noncreatinine chromogens in a nonspecific assay of plasma creatinine. In clinical practice, it is now accepted that, by the time patients have lost one half to two thirds of their normal renal function, as demonstrated by creatinine clearance, it is more reliable and prudent to monitor their subsequent renal function and response to therapeutic initiatives by using radioisotopic markers of glomerular filtration and renal plasma flow.

The methods for creatinine determination most widely used today are based on the Jaffé reaction, first described in 1886. The reaction occurs between creatinine and the picrate ion formed in alkaline medium; a red-orange adduct develops, but despite extensive study, its structure remains uncertain.

The Jaffé reaction has long been known to be nonspecific when applied to the measurement of creatinine in plasma. Among the noncreatinine Jaffé-reacting chromogens are protein, glucose, ascorbic acid, guanidine, acetone, cephalosporins, and α -keto acids such as aceto-acetate and pyruvate. Depending on the method, these "quasi-creatinine" compounds cause an overestimate of plasma "true" creatinine by 0.2 to 0.4 mg/dl. The numerous modifications of Jaffé-reaction methods have had as their chief purpose improvement of specificity for creatinine in plasma; urine has a relatively small content of the same interferents. Protein effect has been eliminated in automated continuous-flow systems by dialysis, in manual methods by a protein precipitation step, or by adding sodium dodecyl sulfate to the reagent in order to form nonreactive protein complexes. Glucose and ascorbic acid have also been complexed, these with borate in a buffered system. Other kind of methods are enzymatic or partially enzymatic assays, based on more specific serial reactions. However these methods are less diffused due to high cost of preparations.

PRINCIPLE OF THE METHOD

Creatinine reacts with picric acid in alkaline environment to form a color complex. Developing of this red color may be followed photometrically at 500-520 nm. The association on surfactant and sodium tetraborate keeps interferences at minimum.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A 2 x 125 ml (liquid) blue cap

Reagent B 2 x 125 ml (liquid) red cap

Composition in the test: picric acid 14 mM, NaOH 0.18 M, sodium tetraborate 10 mM, surfactant.

Standard: creatinine 2 mg/dl - 5 ml

Store all components at 15-25°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micro-pipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Mix 1 part of reagent A with 1 part of reagent B.

Stability of working reagent: ≥ 30 days at 15-25°C, well capped and away from light sources.

Stability of unmixed reagents: up to expiration date on labels at 15-25°C;

Stability since first opening of vials of unmixed reagents: ≥ 60 days at 15-25°C.

PRECAUTIONS

Labelling reagent B: Xi R36/38 Irritating to eyes and skin. S20/21 When using do not eat, drink or smoke.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 After contact with skin wash immediately with plenty of water.

S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

SPECIMEN

Serum, plasma. Urine.

Creatinine is stable 24 hours at 2-8°C. Freeze samples for prolonged storage.

Dilute urine sample 1:100 with deionized water. It could be convenient a slight acidification of urine with HCl.

TEST PROCEDURE

Wavelength:	510 nm (allowed 500 \div 520 nm)
Lightpath:	1 cm
Temperature:	37°C

dispense:	blank	standard	sample
working reagent	2 ml	2 ml	2 ml

incubate at 37°C for 5 minutes

water	200 μ l	-	-
standard	-	200 μ l	-
sample	-	-	200 μ l

Mix, incubate 60 seconds at 37°C, then record absorbance as A_1 . After exactly 60 seconds, record again absorbance as A_2 .

RESULTS CALCULATION

Serum/plasma sample:

$$\text{creatinine mg/dl} = \frac{A_2 - A_1 (\text{sample})}{A_2 - A_1 (\text{standard})} \times 2 (\text{standard value})$$

Random urine sample:

$$\text{creatinine mg/dl} = \frac{A_2 - A_1 (\text{sample})}{A_2 - A_1 (\text{standard})} \times 2 \times 100$$

(standard value and dilution)

24 hours urine sample (creatinine mg/24h):

$$[A_2 - A_1 (\text{sample})] / [A_2 - A_1 (\text{standard})] \times 2 \times 100 \times \text{urine volume}$$

(standard value, dilution factor and diuresis in decilitres)

EXPECTED VALUES

Serum/plasma samples:

Men: 0.7 - 1.2 mg/dl (62 - 105 μ mol/l)
Women: 0.6 - 1.1 mg/dl (53 - 97 μ mol/l)

24h urine: 250 - 750 mg/24h (2.21 - 6.63 mmol/24h)
Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml

with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml

with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 20 mg/dl.

If the value is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 0.1 mg/dl.

Interferences

no interference was observed by the presence of:

hemoglobin ≤ 200 mg/dl

bilirubin ≤ 14 mg/dl

lipids ≤ 600 mg/dl

Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	1.25	0.03	2.60
sample 2	3.87	0.07	1.90

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	1.31	0.04	2.90
sample 2	3.80	0.14	3.80

Methods comparison

a comparison between Chema and a commercially available product gave the following results:

Creatinine Chema = x

Creatinine competitor = y

n = 104

$$y = 0.982x - 0.081 \text{ mg/dl} \quad r = 0.94$$

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

REFERENCES

Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

HU Bergmeyer - Methods of enzymatic analysis, (1987).

MANUFACTURER

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SYMBOLS



for in vitro diagnostic use only



lot of manufacturing



code number



storage at temperature interval



expiration date (year/month)



warning, read enclosed documents



read the directions