CREATININE - JAFFE

(LIQUID)

3 x 50, 3 x 10 ml

RE – ORDER CRE1120

INTENDED USE

This reagent is intended for the kinetic quantitative in vitro determination of creatinine in serum or urine.

TEST SUMMARY

The assay of creatinine has been based on the reaction of creatinine with alkaline picrate. The reaction has been performed on a protein free filtrate. It has long been recognized that the red color formed in this reaction is not absolutely specific for creatinine, thus results obtained are too high in protein free filtrates. However, practically all the chromogenic material in serum appears to be creatinine, therefore the assay in protein free filtrates is adequate for clinical purposes. A number of methods have appeared in the literature which propose to quantitate creatinine by measuring the rate of color formation in the reaction with alkaline picrate. Procedures based on this type of kinetic assay are rapid, do not require pretreatment of the sample, appear to be more specific for creatinine and are readily adapted to automated analysis. Most of the contaminants reacting with the Jaffe reagent produce color at a lower rate than does creatinine. The initial rate of color formation is proportional to the concentration of creatinine in the sample. A more sensitive test for measuring glomerular filtration is the creatinine clearance test. For this test, a precisely timed urine collection (usually 24 hours) and a blood sample are needed. Urine creatinine levels, as well as other markers of dilution, i.e., specific gravity and appearance, are useful as an adjunct to drug abuse testing in determining external dilution or excessive donor hydration.

Ingredients:

Picric Acid Solution

Reactive ingredient:

Picric Acid	22.1 mmol/L

Sodium Hydroxide Solution

Reactive ingredient:

Sodium Hydroxide	0.75 mol/L

Standard Precautions:

Sodium Hydroxide is harmful by inhalation, in contact with skin, and if swallowed. Causes burns. Irritating to eyes, respiratory system and skin.

Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water for at least 10 minutes. If swallowed, seek medical advice immediately and show this container or label. Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.

Intended for in vitro diagnostic use only.

Preparation:

Ready to use.

Storage:

The reagents are stable at room temperature (below 25 °C)

Collection:

No special preparation of the patient is required.

INTERFERING SUBSTANCES:

This Jaffe creatinine method should be considered as a screening assay only, due to the well-known non-specificity of the Jaffe methodology. Interferences noted include non-esterified fatty acids such as palmitic acid, as well as a low frequency of unexplained elevated results associated with high triglycerides. Abnormal and/or suspect results should be confirmed with an alternate assay that is more specific for creatinine, such as an enzymatic methodology. Young has published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including the determination of creatinine. Cephalosporin antibiotic interferences with Jaffe methods for creatinine have been described. When tested at the concentrations indicated below, the following cephalosporin antibiotics spiked into serum containing a level of 0.9 mg/dl creatinine were found to give the amount of interference indicated:

Cefaclor at 100 mg/dL showed an increase of 1.4 mg/dL.

Cefoxitin at 100 mg/dL showed an increase of 4.1 mg/dL.

Cephaloridine at 100 mg/dL showed an increase of 0.4 mg/dL.

Cephalothin at 100 mg/dL showed an increase of 1.1 mg/dL.

The following compounds at the indicated levels gave ≥ 0.3 mg/dL interference as apparent creatinine when added to a normal serum pool.

acetoacetic acid	20 mg/dL
acetone	25 mg/dL
ascorbic acid	10 mg/dL
albumin	3 g/dL
beta-hydroxybutyrate	10 mmol/L
fructose	100 mg/dL
glycerol	30 mg/dL
glucose	500 mg/dL
hemoglobin	1000 mg/dL
pyruvate	0.2 mmol/L
triglycerides (*Intralipid)	1000 mg/dL
urea	1000 mg/dL
uric acid	20 mg/dL

Storage and Handling (20):

Serum: Creatinine in serum is stable for 24 hours at 2 – 8 °C. Freeze for longer storage.

Urine: Creatinine in urine is stable for 4 days at 2 – 8 °C. Freeze for longer storage.

MATERIALS REQUIRED BUT NOT PROVIDED:

Spectrophotometer or colorimeter capable of measuring absorbance at 510 nm.

Matched cuvettes.

Constant temperature incubator set at 30 °C or 37 °C. Use the same temperature for assay of standard, controls and samples.

Distilled or deionized water.

Pipettes to measure water, reagent(s) and samples.

Instructions:

Prepare a working reagent by combining 5 parts Picric Acid Solution with 1 part Sodium Hydroxide Solution. Mix well.

The working reagent is stable for 1 month when stored protected from light at room temperature (below 25 °C).

Do not mix working solutions of different age. Do not reuse bottles.

Wavelength: 510 nm.

Set the spectrophotometer to 0 absorbance with water.

*Intralipid is a registered trademark of Kabivitrum, Inc., Clayton, NC.

Bring working reagent, standard, controls and samples to incubation temperature (30 or 37 °C).

Standard	Sample	
Working reagent	1.0 mL	1.0 mL
Standard	**0.1 mL**	**—**
Sample	—	0.1 Ml

Mix gently.

Incubate at the selected incubation temperature. Read the absorbance of the standard and samples at exactly 40 and 100 seconds. Determine ΔA/minute of the reaction.

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CALIBRATION

This assay requires the use of a standard or calibrator. Use the standard provided with the reagent or other commercially available creatinine standards or calibrators. This assay should be calibrated in accordance with the instrument manufacturer's specifications. Calibration stability is depended upon the instrument performance and the proper storage of the reagents. Re-calibration is recommended at anytime, should one of the following occur. Change in the reagent lot number. Preventative maintenance is performed on the analyzer. A critical element of the analyzer is replaced. Control material results have shifted or are out of range and the use of a freshly reconstituted vial of control does not correct the situation. It is recommended that each laboratory establish its own procedures for corrective action if calibration is not acceptable.

QUALITY CONTROL

Controls are recommended to monitor the performance of the assay, providing a constant screening of the instrument, reagents and techniques. Raichem Assayed Control, Level 1 (Order No. 83082) and Assayed Control, Level 2 (Order No. 83083) are recommended for this purpose. It is recommended that each laboratory establish an acceptable range of Creatinine values by repeat analysis. Quality control material should be assayed when:

There is a change in the reagent lot number.

The assay has been calibrated.

Preventative maintenance is performed on the analyzer.

A critical element of the analyzer is replaced.

The individual laboratory requirements specify that quality control material is to be run. It is recommended that each laboratory establish its own control schedule and procedures for corrective action if controls do not recover within the specified tolerances.

calculations

$$\frac{\Delta A/\text{min. Sample}}{\Delta A/\text{min. Standard}} \times C_{ST} = \text{mg/dL creatinine in the sample}$$

Where C_{ST} = concentration of standard or calibrator

Conversion Factor = mg/dL x 88.4 = µmol/L

If the sample was urine and it was diluted 1:20, multiply the results by 20 to obtain the mg/dL in the original specimen.

Sample Calculation:

If the ΔA/minute of a serum sample was 0.028 and the ΔA/minute of a 2.0 mg/dL standard was 0.058:

$$\frac{0.028}{0.058} \times 2.0 = 0.96 \text{ mg/dL creatinine in the serum sample.}$$

If the ΔA/minute of a urine sample, diluted prior to assay 1:20, was 0.055 and the ΔA/minute of a 2.0 mg/dL standard was 0.058:

$$\frac{0.055}{0.058} \times 2.0 \times 20 = 38 \text{ mg/dL creatinine in the urine sample.}$$

Creatinine Clearance

The creatinine clearance is calculated using the following formula:

$$\text{Creatinine clearance (ml/min)} = \frac{U}{P} \times V \times \frac{1.73}{A}$$

where:

U =Concentration of creatinine in the urine

P =Concentration of creatinine in the serum (or plasma)

V =Volume of urine in ml/min

A =Body surface area in square meters

1.73 = Average body surface in square meters

The patient's body surface may be calculated using the following formula or obtained more conveniently from an available nomogram (5).

$$\log A = (0.425 \log W) + (0.725 \log H) - 2.144$$

where:

A =The body surface area in square meters

W =The weight of the patient in kg

H =The height of the patient in cm

Total creatinine in 24 hour urine:

The concentration of creatinine in 24-hour urine is determined as follows:

$$\frac{\text{mg/dL creatinine x liters urine/24 hours}}{100} = \text{concentration in g /24 hours}$$

REFERENCE RANGE

THE REFERENCE RANGE FOR SERUM CREATININE IS AS FOLLOWS (20):
0.40 – 1.40 mg/dl

It is highly recommended that each laboratory establish its own reference range.

REAGENT PERFORMANCE

Linearity: 5 mg/dL.

Accuracy: Employing as reference a commercial reagent based on the same procedure (Sclavo) at 30 °C in 73 samples ranging in concentration from 0.6 mg/dL to 5.77 mg/dL, the correlation coefficient was 0.997 and the regression equation was y = 1.022x + 0.010. Precision: Assays were run, following the procedure described above, on two serum pools. Results were:

Run to Run		
Mean (mg/dL)	0.77	3.73
SD	0.05	0.16
CV (%)	6.3	4.3
N (13 assays each run)	3	3
Within Run		
Mean (mg/dL)	0.77	3.77
SD	0.03	0.11
CV (%)	3.9	2.8
N	13	13

Sensitivity: 1 mg/dL = 0.029 ΔA/min at 30 °C.

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