POINTE SCIENTIFIC, INC.
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Creatinine Reagent Set

Intended Use
For the quantitative determination of creatinine in serum. For *in vitro* diagnostic use only.

Clinical Significance
Creatinine assays are most frequently performed to aid in the determination of renal function.

Method History
In 1886, Jaffe[1] described a method for the determination of creatinine involving a protein free filtrate and a reaction with picric acid in alkaline solution. Although several methods have been described since then, the classic Jaffe reaction is still the most widely used. The Jaffe reaction is subject to interferences by a number of substances, including protein and glucose.[2,3,4] Modifications of the procedure have been developed to combat the drawbacks.[5] The kinetic procedures[6] have become popular because they are fast, simple and avoid interference. The present method is based on a modification of the above procedure, incorporating a surfactant and other ingredients to minimize protein and carbohydrate interferences.

Principle
Creatinine + Sodium Picrate ---(Alkali)---➢ Creatinine-picrate complex (yellow-orange)

Creatinine reacts with picric acid in alkaline conditions to form a color complex that absorbs at 510 nm. The rate of formation of color is proportional to the creatinine in the sample.

Reagents
Creatinine R1 Reagent: Alkaline Buffer
Creatinine R2 Reagent: Picric Acid 40mM, Surfactant

Reagent Preparation
Reagents are ready to use for some systems. If a working reagent is necessary, combine five volumes of R1 and one volume of R2 reagent, mix. See instrument application instructions.

Reagent Storage and Stability
1. Both reagents are stored at room temperature.
2. Combined (working) reagent is stable for up to one month at room temperature if tightly capped.

Reagent Deterioration
Do not use if:
1. The reagent is cloudy (contaminated).
2. The reagent fails to achieve assigned values on fresh control sera.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Picric Acid is a strong oxidizing agent. Avoid contact with skin. WIPE ANY SPILLAGE, SINCE EVAPORATED PICRIC ACID IS EXPLOSIVE.
3. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories", 2nd Ed. 1988, HHS Publication No. (CDC) 88-8395.

Specimen Collection and Storage
1. Serum is recommended.
2. Creatinine in serum is stable for twenty-four hours at refrigerated temperatures (2-8°C) and for several months when frozen (-20°C) and protected from evaporation and contamination.
3. 24-hour urine specimens must be preserved with 15 grams of boric acid.
4. Specimen collection should be carried out in accordance with NCCLS M29-T2.[7] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences
A number of substances affect the accuracy of creatinine. See Young, et al.[8]

Materials Provided
1. Creatinine R1 Reagent
2. Creatinine R2 Reagent

Materials Required but not Provided
1. Accurate pipetting devices
2. Timer
3. Test tubes/rack
4. Spectrophotometer with a temperature controlled cuvette
5. Heating Block (37°C).

Procedure (Automated)
Refer to specific instrument application instructions.

Wavelength:	510nm
Assay Type:	Initial Rate
Sample/Reagent Ratio:	1:20
Reaction Direction:	Increasing
Temperature:	37°C
Lag Time:	60 sec.
Read Time:	60 sec.
Low Normal:	0.40
High Normal:	1.40

Application Parameters for various automated instruments are available. Please contact the Technical Service Department for specific information.

Procedure (Manual)
1. Prepare working reagent according to instructions.
2. Set the spectrophotometer cuvette temperature to 37°C.
3. Pipette 1.0 ml of working reagent into each tube.
4. Zero spectrophotometer with the reagent blank at 510 nm.
5. Add .05 ml (50ul) of sample to reagent, mix and immediately place into cuvette.
6. After exactly sixty seconds read and record the absorbance (A_1)
7. At exactly sixty seconds after the A_1 reading, again read and record the absorbance (A_2), i.e. the time elapsed between A_1 and A_2 is sixty seconds.
8. Calculate the change in absorbance (Δ Abs/min) by subtracting (A_2-A_1). See "Calculations".

NOTE: If the spectrophotometer in use requires a volume greater than 1.0 ml for accurate reading, use 0.20 ml (200 ul) sample to 3.0 ml reagent. Perform as above.

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Limitations
Samples with values above 25 mg/dl should be diluted 1:1, re-assayed and results multiplied by two.

Calibration
Use an NIST-traceable creatinine standard (2.5mg/dl) or serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculation
The creatinine value of the unknown is determined by comparing its absorbance change with that of a known standard.

$$\text{Mg/dl} = \frac{\Delta \text{ Abs (Unknown)}}{\Delta \text{ Abs (Standard)}} \times \text{Concentration of Std. (mg/dl)}$$

Where: Δ Abs. = Absorbance change between readings (A_2-A_1)

Sample Calculation
If: Δ Abs/Unknown = 0.02
Δ Abs/Standard = 0.05
Conc. of Standard = 2.5 mg/dl

Then: $\frac{0.02}{0.05} \times 2.5 = 1.0$ mg/dl creatinine

Quality Control
The integrity of the reaction should be monitored by use of normal and abnormal control sera with known creatinine values. These controls should be run at least with every working shift in which creatinine assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values
0.40 – 1.40 mg/dl

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

Performance
1. Assay Range: 0.1-25.0 mg/dL
2. Correlation: A patient correlation of 126 specimens over the range of about 0.4 to 11.3 mg/dL creatinine yielded y=1.09 x + 0.02, r^2 = 0.998 and S.E. = 0.22.
3. Precision: Precision studies were performed following a modification of the guidelines which are contained in the NCCLS document EP5-T2.[9]

Within Day			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
1.4	0.03	2.2	1.4	0.05	3.6
7.1	0.09	1.3	7.0	1.10	1.6

References

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7. NCCLS document "Protection of Laboratory Workers form Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
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