Creatinine Jaffé Fluid 5+1

Kinetic in vitro assay for the quantitative determination of creatinine in human serum, plasma and urine

**CALIBRATION**

S1: 0.9% NaCl or distilled/deionized water

S2: Creatinine standard 5ml 88 83 98

**QUALITY CONTROL**

Control Serum N 6x5ml 88 41 48B

Control Serum P 6x5ml 88 46 85B

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**SUMMARY**

In muscle metabolism, creatinine is synthesized endogenously from creatine and creatine phosphate. Under conditions of normal renal function, creatinine is excreted by glomerular filtration. Creatinine determinations are performed for the diagnosis and monitoring of acute and chronic renal disease as well as for the monitoring of renal dialysis. Creatinine concentrations in urine can be used as reference values for the excretion of certain analytes (albumin, α-amylase).

**INTENDED USE**

Kinetic in vitro assay using rate-blanking and compensation for the quantitative determination of creatinine in human serum, plasma and urine.

**TEST PRINCIPLE**

Kinetic colorimetric assay

Sample and addition of R1 (sodium hydroxide)

Addition of R2 (picric acid) and start of reaction:

\[
\text{creatinine} + \text{picric acid} \xrightarrow{\text{alkaline solution}} \text{creatinine} - \text{picric} - \text{acid} - \text{complex}
\]

In alkaline solution, creatinine forms a yellow-orange complex with picrate. The color intensity is directly proportional to the creatinine concentration and can be measured photometrically. Assays using rate-blanking minimize interference by bilirubin. Serum and plasma samples contain proteins which react non-specifically in the Jaffe method. Serum and plasma results must be reduced by 0.3 mg/dl (26 µmol/l) to obtain accurate values. This correction causes a measurement error of \(\leq 1\%\) in urine specimens because these do not contain non-specific proteins.

**NOTES**

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents.

Warning: Bottle 1 contains NaOH-solution, corrosive. Bottle 2 contains priced acid; hazardous, toxic. In case of contact, flush affected areas with copious amounts of water. Get immediately medical attention in case of contact with eyes or if ingested.

**LIMITATIONS - INTERFERENCE**

Criterion: Recovery within ±10% of initial value.

Icterus: No significant interference up to an index I of 10 (approximate bilirubin: 10 mg/dl)

Hemolysis: No significant interference up to an index H of 1100 (approximate haemoglobin concentration: 1100mg/dl)

Lipemia (Intralipid): No significant interference up to an index L of 1074 (approximate triglycerides concentration: 2148 mg/dl)

Negatively biased results have been reported due to a temporary production of turbidity in the early stages of the
reaction. This effect correlates with increased triglycerides in the serum sample, but does not correlate as well with sample Lipemic Index values. The effect disappears after overnight storage.
Do not use creatinine Jaffé for the assay of creatinine in haemolysed samples from neonates, because foetal hemoglobin (HbF) can cause negative values.

**MEASURING/REPORTABLE RANGE**

*Serum/plasma:* 0.1-25 mg/dl (8.8 - 2210 µmol/l)  
*Urine:* 0.1-350 mg/dl (8.8-30940 µmol/l)

Determine samples with higher concentrations via the rerun function. On instruments without rerun function, manually dilute samples with 0.9% NaCl or distilled/deionized water (e.g. 1+1). Multiply the result by the appropriate dilution factor (e.g. 2)

**EXPECTED VALUES**

Expected values according to Mazzachi, Peake and Ehrhardt:

*Serum/plasma (adults)*
- Men: 0.70 - 1.20 mg/dl (62 - 106 µmol/l)
- Women: 0.50 - 0.90 mg/dl (44 - 80 µmol/l)

*1st morning urine (adults)*
- Men: 30 - 259 mg/dl (3450 - 22900 µmol/l)
- Women: 28 - 217 mg/dl (2470 - 19200 µmol/l)

*24-hour urine*
- 600-2000 mg/24 h (5-18 mmol/24 h) corresponding to 40-133 mg/dl** (3300-12 000 µmol/l**)  
**Based on a urine volume of 1.5 l/24 h

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the creatinine results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**ANALYTICAL SENSITIVITY (LOWER DETECTION LIMIT)**

Detection limit: 0.1mg/dl (8.8 µmol/l)  
The lower detection limit represents the lowest measurable creatinine concentration that can be distinguished from zero.

**IMPRECISION**

*Serum*
Reproducibility was determined using controls. The following results were obtained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean mg/gl</th>
<th>SD mg/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.21</td>
<td>0.025</td>
<td>2.07</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3.86</td>
<td>0.076</td>
<td>1.97</td>
</tr>
<tr>
<td>Sample 3</td>
<td>4.03</td>
<td>0.074</td>
<td>1.84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>Mean mg/gl</th>
<th>SD mg/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.34</td>
<td>0.021</td>
<td>1.57</td>
</tr>
<tr>
<td>Sample 2</td>
<td>4.14</td>
<td>0.039</td>
<td>0.94</td>
</tr>
<tr>
<td>Sample 3</td>
<td>4.17</td>
<td>0.028</td>
<td>0.67</td>
</tr>
</tbody>
</table>

**METHOD COMPARISON**

A comparison of the AXIOM Creatinine Jaffé Fluid 5+1 (y) with a commercial obtainable assay (x) gave the following result (mg/dl):  
y = 1.011 x – 0.0093; r = 0.971

**REAGENT CONCENTRATION**

*R1:*  
Sodium hydroxide 0.20 mol/l

*R2:*  
Picric acid 25 mmol/l

**PREPARATION AND STABILITY**
R1: Ready for use
R2: Ready for use

Unopened kit components: up to the expiration date at +15°C to +25°C.

Absorption of atmospheric CO₂ by the opened reagent bottle R1 leads to impaired calibration stability. The creatinine Jaffe method was standardized against GC/MS as a reference method.

Onboard stability:
- R1: 28 days opened and refrigerated on the analyser
- R2: 56 days opened and refrigerated on the analyser
- R1-R2-mixture: 28 days at +2°C to +8°C
- 8 hours at +15°C to +25°C

SPECIMEN:
Serum, heparinized or EDTA- plasma.

Stability: 7 days at +2°C to +8°C  Freeze for long-term storage.

Urine

Stability: 4 days at +2°C to +8°C  Freeze for long-term storage.

Collect urine without additives.

TESTING PROCEDURE

Materials provided
- Working solutions as described above

Additional materials required
- Calibrators and controls as indicated below
- 0.9% NaCl

Manual procedure:

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Hg 492/500 nm; Cd 509 nm</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>+25°C / +37°C</td>
</tr>
<tr>
<td>Cuvette</td>
<td>1 cm light path</td>
</tr>
<tr>
<td>Zero adjustment</td>
<td>against reagent blank</td>
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</tbody>
</table>

Mix R1 and R2 in the ratio of 5+1.

<table>
<thead>
<tr>
<th>Standard / Calibrator</th>
<th>Sample</th>
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<tbody>
<tr>
<td>R1-R2-mixture</td>
<td>1000 µl</td>
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<tr>
<td>Standard</td>
<td>200 µl</td>
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<tr>
<td>Sample</td>
<td>---</td>
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Mix and incubate 60 seconds at +25°C or 30 seconds at +37°C, then read absorbance (A₁(sample)) and standard (A₁_standard). Repeat reading after exactly 5 minutes at +25°C or 3 minutes at 37°C (A₂).

Determine the absorbance change as:

\[ \Delta A_{\text{sample}} = [A_2 (\text{sample}) - A_1 (\text{sample})] \]

\[ \Delta A_{\text{standard}} = [A_2 (\text{standard}) - A_1 (\text{standard})] \]

and use this for the calculation.

Calculation:

\[ \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{standard conc.} = \text{Creatinine conc. (mg/dl)} \]

CALIBRATION FREQUENCY

Two-point calibration is recommended:
- every 14 days if the reagent bottles are onboard the analyzer for more than 14 days.
- after reagent bottle change if the previous reagent bottles were onboard the analyzer for more than 14 days.
- after reagent lot change
- as required following quality control procedures

DISPOSAL
Please note the legal regulations.

**LITERATURE**


**AXIOM Product range Clinical Chemistry**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Ions</th>
<th>Other Metabolites</th>
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<tbody>
<tr>
<td>Acid Phosphatase</td>
<td>Ammonium fluid</td>
<td>Bilirubin T/D</td>
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<tr>
<td>Alkaline Phosphatase</td>
<td>Copper fluid</td>
<td>Creatinine fluid</td>
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<td>Chloride fluid</td>
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<td>Triglycerides fluid</td>
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<tr>
<td></td>
<td>Protein Total fluid</td>
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</table>

06/09 M/kd