Intended Use
System reagent for the quantitative determination of Creatinine concentration in human urine as an indicator of adulteration on the Beckman Coulter AU2700/5400 analyzer. This assay is for forensic/toxicology purposes only.

Summary
Creatinine is an end product of glycine and arginine metabolism. Urine creatinine measurements along with specific gravity and visual appearances, are useful in the evaluation of specimen adulteration in urine samples submitted for drugs of abuse testing.

Methodology
The Urine Tox Creatinine procedure for use on the AU2700/5400 analyzer is a kinetic modification of the Jaffe procedure. Creatinine reacts with alkaline picrate to form a red colored complex. The rate of change in absorbance is measured bichromatically at 520/800 nm and is proportional to the creatinine concentration in the sample.

System Information
For AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:
- Picric acid 1.3 mmol/L
- Sodium hydroxide 110 mmol/L
- Also contains preservatives.

Precautions
1. For in-vitro forensic/toxicology use.
2. Creatinine R1 reagent contains Picric Acid which is poisonous. Dry picric acid explodes if rapidly heated or subjected to percussion. Dilute any spills with water and wipe up immediately. The reagent will also stain skin and clothing. In case of external contact, flush with copious amounts of water. For ingestion or eye contact, seek medical attention.
3. Creatinine R2 reagent is corrosive. DO NOT PIPETTE BY MOUTH. Avoid contact with skin or eyes. In case of external contact, flush with copious amounts of water. For ingestion, seek immediate medical attention.

Preparation of Reagents
The R1 and R2 reagents are ready for use and may be placed directly on board the instrument.

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 15 - 25°C.
2. The opened and working reagents are stable for 14 days when stored in the refrigerated reagent compartment of the AU2700/5400 analyzer.

Indications of Deterioration
Turbidity in the unopened liquids and working reagent may indicate decomposition and warrant discontinuance of use.

Specimen Collection and Preparation
Clear, freshly obtained urine specimens are the recommended samples. Highly turbid or particulate specimens should be centrifuged prior to testing. No special additives or preservatives are required.

Sample Storage and Stability
Urine creatinine is stable for up to 5 days when stored at 2-8°C. Frozen samples (-20°C) are stable indefinitely. Frozen samples must be thawed and thoroughly mixed prior to testing.

Interfering Substances
Drugs and other adulterants may affect Urine Tox Creatinine determinations. Refer to Young for a compilation of reported interferences with this test. Results of studies conducted on the AU2700/5400 show that the following substances interfere with the Urine Tox Creatinine procedure.

The criteria for no significant interference is recovery within 10% of the initial value.
- Ascorbate: No significant interference up to 250 mg/dL Ascorbate
- Bilirubin: No significant interference up to 20 mg/dL Bilirubin
- Glucose: No significant interference up to 5000 mg/dL Glucose

Procedure
Materials Provided
Beckman Coulter AU2700/5400 Urine Tox Creatinine reagent

Materials Required But Not Provided
Beckman Coulter AU2700/5400 analyzer
Sample cups (Catalog No. AU1063)
Syva® CR PERFECT™ Calibrators: 3T129UL (Negative Calibrator/Control), 3T249UL (2.0 mg/dL Calibrator), 3T139UL (20 mg/dL Calibrator), 3T149UL (100 mg/dL Calibrator)
Urine Tox Creatinine

Suggested Analytical Parameters
Refer to the Methodology Section located in the respective AU analyzer’s User’s Guide.

Stability of Final Reaction Mixture
The AU2700/5400 analyzer automatically computes each determination at the same time interval at 37°C.

Calibration
The frequency of calibration is every 7 days. Calibration of this Creatinine procedure is accomplished by use of the Syva cR PERFECT Calibrator material, which is traceable to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 909a-2.

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed and there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
A low and high urine toxicology control should be analyzed routinely with each run of unknown samples.

Results
Automatically printed out for each sample in mg/dL at 37°C.

Dynamic Range
The AU2700/5400 Urine Tox Creatinine procedure is linear from 1.0 to 100.0 mg/dL. Samples exceeding the dynamic range of the assay may be diluted with isotonic saline and re-assayed. The results obtained must be multiplied by the dilution factor to obtain the correct concentration for the undiluted sample.

Expected Values
In conjunction with specific gravity and other guidelines outlined by SAMHSA, the following creatinine concentrations indicate substituted and dilute urine:
- Substituted: less than 2.0 mg/dL
- Dilute: greater than or equal to 2.0 mg/dL and less than 20.0 mg/dL

Specific Performance Characteristics
The following data was obtained using the AU2700/5400 Urine Tox Creatinine reagent on an Beckman Coulter AU2700/5400 analyzer according to established procedures. Results obtained in individual laboratories may differ.

Precision
Estimates of precision, based on CLSI recommendations, are consistent with typical performance. Assays of urine control material were performed and the data reduced following CLSI guidelines above:

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<th>AU2700/5400 N = 80</th>
<th>Within run</th>
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Method Comparison
A comparison of this Urine Tox Creatinine method (Method 1) versus commercially available Urine Toxicology Creatinine method (Method 2) was run on the AU5400 utilizing 97 patient urine samples.

The resulting data is as follows:
- Correlation Coefficient: 0.9958
- Regression Equation: Method 1 = (Method 2 x 1.004) + 0.47
- Range: 1.90 - 97.40 mg/dL

Lowest Quantifiable Level
The lowest level of Creatinine that can be quantified by this method with less than 20% CV is 0.55 mg/dL.

Lowest Detectable Level
The lowest level of Creatinine that can be statistically distinguished from zero with this method is 0.16 mg/dL.

Sensitivity
Typical change in absorbance for 1 mg/dL of Creatinine is 0.9 mAbsorbance per minute.

References
2. SAMHSA. Mandatory guidelines for federal workplace drug testing programs. Substance Abuse and Mental Health Services Administration (SAMHSA), effective Nov 1, 2004.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA