POINTE SCIENTIFIC, INC.
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Urea Nitrogen (BUN) (Berthelot/Colorimetric)

Intended Use
For *in vitro* diagnostic use in the quantitative colorimetric determination of urea nitrogen in serum.

Clinical Significance[1]
Urea, an end product of protein metabolism, is excreted by the kidney. Blood urea nitrogen (BUN) varies directly with protein intake and inversely with the rate of excretion of urea.

BUN levels are elevated under the following conditions:

1. Renal insufficiency: acute and chronic nephritis, acute renal failure (tubular necrosis), urinary tract obstruction.
2. Increased nitrogen metabolism associated with diminished renal blood flow or impaired renal function: dehydration from any cause, gastrointestinal bleeding, with a combination of increased protein absorption from digestion of blood, plus decreased renal blood flow.
3. Decreased renal blood flow: shock, adrenal insufficiency, occasionally congestive heart failure.
4. Many of the antibiotics that impair renal function; guanethidine, methyldopa, indomethacin, isoniazid, propranolol, and potent diuretics (decreased blood volume and renal blood flow).

BUN levels are decreased under the following conditions:

1. Hepatic failure
2. Nephrosis not complicated by renal insufficiency
3. Cachexia (general poor health with weakness and malnutrition).

Test History and Principle[2]
The Berthelot reaction, in which ammonia reacts with hypochlorite, phenol, a catalyst, and alkali to produce a stable blue complex (indophenol) has been known for over 100 years but only relatively recently used in a method for serum urea. The use of sodium nitroferricyanide was introduced in 1962 and the substitution of salicylate for phenol was introduced in 1967.

This procedure is based upon a modified Berthelot reaction wherein urease hydrolyzes urea to ammonia and carbamic acid. Carbamic acid spontaneously decomposes into ammonia and carbon dioxide. Ammonia reacts with salicylate, nitroferricyanide and an alkaline solution of hypochlorite to yield a blue-green chromophore which is measured photometrically and is proportional to the amount of urea in the sample.

Reagents
The following reagents should be stored at 2-8°C. and can be used until the expiration date indicated on the individual bottle:

ENZYME REAGENT
A solution of urease buffered at pH 6.7 – 6.8 also containing preservative and stabilizers. Avoid contamination. Store at 2-8°C. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended.

COLOR REAGENT
A solution containing sodium salicylate, sodium nitroferricyanide and preservative. Avoid contamination. Store at 2-8°C. Do not ingest. Exercise the normal precautions required for the handling of all laboratory reagents. Do not pipette by mouth.

BASE REAGENT
A solution containing sodium hydroxide and sodium hypochlorite. Avoid contamination. Store at 2-8°C. CORROSIVE! In case of contact, flush affected area with large amounts of water. Seek medical attention. Do not pipette by mouth.

STANDARD (25 MG/DL)
A solution containing urea equivalent to 25 mg/dl with preservative. Avoid contamination. Store at 2-8°C. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended.

Reagent Deterioration
Do not use if:
Appearance of turbidity, visible mold growth, discoloration, or crystal formation that will not readily dissolve

Instrument Requirements
Any instrument capable of reading absorbance accurately with a sensitivity of 0.005 absorbance at 630 nm may be used. The bandwidth should be less than 10 nm, stray light of 0.5% or less, and the wavelength accuracy within 5 nm.

Specimen[3]
Serum is recommended for the assay. Plasma may also be used, provided that the anticoagulant used contains neither ammonium nor fluoride salts. Sodium, potassium, or lithium salts or heparin, EDTA, or oxalate are satisfactory anticoagulants.

Urea in serum is stable up to 24 hours at room temperature, for at least several days at 4-6°C, and for at least 2-3 months when frozen.

Materials Provided
Enzyme reagent, color reagent, base reagent, standard (25mg/dl)

Materials Required but not Provided
1. Accurate pipetting devices
2. Timer
3. Cuvettes
4. Spectrophotometer
5. 37°C heating bath
6. Controls

Procedure
1. Transfer 0.5 ml of COLOR RGT to vials labeled: UNKNOWN, CONTROL, STANDARD, BLANK.
2. Add 0.010 ml (10ul) of sample to its corresponding vial.
3. Add 0.5 ml of ENZYME RGT to all vials, mix gently, and incubate at 37°C for five minutes. (Alternative: React for 10 minutes at room temperature 2-26°C).
4. Add 2.0 ml of BASE RGT, mix and incubate at 37°C for 5 minutes. (Alternative: React for 10 minutes at room temperature 2-26°C).
5. Set the wavelength of the photometer at 630nm and zero the photometer with the BLANK. Read and record the absorbances of all vials and proceed to the Calculation with Example below.

NOTE: For a direct read-out instrument, set read out to concentration of Standard (25 mg/dl). Read the Unknown concentration directly.

Procedure Notes
1. If a sample exceeds the linearity of the test, make a five-fold dilution of sample with ammonia-free reagent grade water and re-run assay; multiply result by 5.
2. If urine is to be assayed; we recommend the use of a diacetyl monoxime based method.

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Calculations

Where: A = absorbance, U = UNKNOWN, S = STANDARD, C = concentration:

$$\frac{A(U)}{A(S)} \times C(S)\ mg/dl = C(U)\ mg/dl$$

Example: A(U) = 0.31, A(S) = 0.48, C(S) = 25 mg/dl
Then:

$$\frac{0.31}{0.48} \times 25\ mg/dl = 16\ mg/dl$$

Endpoint Stability

The final colored reaction product is stable for at least 30 minutes.

Procedure Limitations

Sources of error usually are limited to ammonia contamination of glassware, reagents or atmosphere and fluoride or other potent enzyme inhibitors (i.e. mercury). For a review of drug and disease effects on urea nitrogen values and methods, see references 4 and 5.

Quality Control

Quality control sera should be used routinely to monitor test precision.[7] Refer to the manufacturer's package insert for analyte stability and acceptable limits.

Expected Values[3]

Healthy Ambulatory Adults …….. 7-18 mg/dl
These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

Performance

Linearity: The method is linear for urea nitrogen values up to 45 mg/dl.

Sensitivity: Typically, 0.001 A = 0.05 mg/dl in a 1-cm lightpath at 630 nm.

Accuracy: RECOVERY STUDIES (in triplicate)

Added	Found	%Recovery
10	10.08	100.80
30	30.11	100.37
50	49.87	99.74

Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
14.5	0.41	2.83	14.7	0.52	3.54
50.1	1.63	3.25	51.6	1.96	3.80

References

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3. Tietz, N.W., textbook of Clinical Chemistry, W.B. Saunders Co., Philadelphia, p.1270-1271 (1986).
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