

UREA (BERTHELOT)

INTENDED USE

Bioline Urea Berthelot reagent is used for the quantitative determination of Urea in human serum used in routine examination and monitoring of therapy and relapses.

CLINICAL SIGNIFICANCE

Urea is the principle waste product of protein catabolism. It is synthesized in the liver from ammonia which is produced as a result of the deamination of amino acids. Normally, urea nitrogen in the blood comprises only about 45% of the non-protein nitrogen. The importance of urea nitrogen determination is its value as an indicator of liver and kidney functions. Decreases in Urea are seen with nephritis, acute liver destruction, amyloidosis and pregnancy.¹ Increases in Urea are encountered with acute and chronic nephritis, intestinal and urinary obstruction, uremia, metallic poisoning, pneumonia, Addison's disease, peritonitis, surgical shock and cardiac failure.

Urea + H₂O $\xrightarrow{\text{Urease}}$ 2NH₃ + CO₂

NH₃ + Salicylate + Hypochlorite $\xrightarrow{\text{Nitroprusside}}$ 2 - 2 - Dicarboxy Indophenol.

METHOD AND PRINCIPLE

Urease catalyses the conversion of Urea to Ammonia and Carbondioxide. The ammonia released reacts with a mixture of Salicylate, Hypochlorite and Nitroprusside to yield a blue-green colored compound (Indophenol). The intensity of color produced is proportional to the concentration of urea in the sample and is measured photometrically at 578 nm

REAGENT COMPOSITION

Urease 40,000 U/l; Sodium Hypo chlorite 30 mMol/l ;NaOH 380 mMol/l ;Sodium Salicylate 50 mmol/l ; Sodium Nitroprusside 28 m mol/l Activators and Stabilizers

1. Urea Standard (40 mg/dL)

WARNINGS AND PRECAUTIONS

1. Reagent R₃ is an alkaline solution. Avoid contact with skin. Flush with plenty of water if contact occurs . Don't pipette by mouth.
2. Avoid contamination with ammonium.
3. Don't expose the reaction medium to direct strong light.
4. Reagent R₁ contain sodium azide as a preservative. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.

WORKING REAGENT PREPARATION

1. The working reagent is prepared by mixing 1 volumes of R₂ with 20 volume of R₁ in a disposable container.
Example: 0.050 ml R₂ + 1.0 ml R₁.
This working reagent is stable for 30 days at 2-8 deg c
2. Urea Standard is ready to use.

REAGENT STORAGE AND STABILITY

Reagent R₁, R₂ and R₃ are liquid, ready to use. All reagents are stable up to expiry date given on the label when stored at +2 to 8 deg c.

REAGENT DETERIORATION

Discard if either appears cloudy or contains particulate matter. The working reagent should be discarded if the initial absorbance, read against distilled water at 578nm, is above 0.200 absorbance.

SPECIMEN COLLECTION AND STABILITY

1. Serum is the recommended sample.
2. EDTA, citrate, Na-Heparin, Na-Fluoride or oxalate

anticoagulants could be used.

3. Anticoagulants containing ammonium or fluoride salts should not be used.
4. All material coming in contact with the sample must be free of ammonia and heavy metals.
5. Urea in serum is reported stable for seventy-two hours refrigerated at +2 to +8 °C. Unrefrigerated sera should be used within eight hours.
6. Fresh urine: dilute urine (1+20) with double distilled water and multiply result by 21.

INTERFERENCES

Fluoride and ammonia cause interference with the Urea assay. Blood collected in tubes containing ammonium heparinate should NOT be used. Young, et al provide a list of drugs and other substances that interfere with the determination of Urea.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength 578 nm

Temperature 37°C

	Blank	Cal/Std	Sample
Dist water	5 µl	-	-
Calibrator/Standard	-	5 µl	-
Sample	-	-	5 µl
Working Reagent	500 µl	500 µl	500 µl
Mix Incubate for 3 min at 37 deg c or 6 min at 20-25 deg c			
Reagent R ₃	500 µl	500 µl	500 µl
Mix incubate for at least 5 min at 37 deg c or 10 min at 20-25 deg c			
Read absorbance (A). The final color is stable for 1 hour.			

CALCULATIONS

Serum

$\frac{A_{\text{Sample}}}{A_{\text{Std}}} \times \text{Concn of Std} = \text{Urea (mg/dl)}$

A Std

Urine

$\frac{A_{\text{Sample (Diluted)}}}{A_{\text{Std}}} \times \text{Concn of Std} \times \text{Dilution factor} = \text{Urea (mg/dl)}$

A Std

Multiply the above result with 21 to get the qty of urea/ litre then finally multiply with the Volume of urine collected in litres.

CALIBRATION

The procedure is calibrated with the standard solution, which is included with each series of tests. Its absorbance is used to calculate the results.

LIMITATIONS

If the urea value exceeds 250 mg/dL, the specimen should be diluted 2-fold (1+1) with distilled water, the assay repeated and results multiplied by the dilution factor of 2. Urea values for neonatal patients have not been established with this procedure.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established urea values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

Serum : < 50mg/dl

Urine/24 hr : 26-43 g/24 hr

This range should serve only as a guide line. It is ultimately the responsibility of the laboratory to establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

PERFORMANCE CHARACTERISTICS

1. Comparison: A group of 40 sera ranging in urea values from 5.9-108.5 mg/dL was assayed by the described urea method and by a similar commercially available urea berthelot reagent. Comparison of the results yielded a correlation coefficient of 0.994 and the regression equation as $y=0.962x-0.721$.
2. Precision: Within-run precision was established by 20 assays on two different levels of commercial serum controls. Total precision values were obtained by assaying two commercial controls for five consecutive days.
3. Linearity: 250 mg/dL.

General Technical Parameters

Mode	End Point
Wavelength (Filter)	578 nm
Reaction Direction / Type	Increasing
Reagent Blank	Yes
Sample Vol.	5 μ L
Working Reagent Vol.	(500 μ L buffer + 500 μ L Chromogen)
Incubation Time	3+5 min at 37 deg
Reagent Blank Abs.(Max)	NMT 0.200 Abs
Calibration Method	1- Point
Standard (Conc.)	40 mg/dL
Linearity	250 mg/dL
Decimal Places	1
Temp.	37 deg c
Unit	mg/dL
Ref. Low (Male / Female)	10 mg/dL
Ref. High (Male / Female)	50 mg/dL

REFERENCES

1. Sampson, E.J., Baird, M.A., Burtis C.A., et al.: A coupled-enzyme equilibrium method for measuring urea in serum: Optimization and evaluation of the AACC Study Group on urea candidate reference method. Clin Chem., 26, 816-826, 1980.
2. Henry, R.J., Clinical Chemistry Principles and Techniques, 2nd Edition, Harper and Row, Hagerstown, NY, (1974), p.511.
3. Tietz, N.W., Text book of Clinical Chemistry, W.B. Saunders Company, Philadelphia, PA, (1986), p.1270-1271.
4. Young, D.S., 3rd Ed., AACCPress, Washington DC (1990).