TRUEchemie Urea Test Kit (Berthelot - End Point)

for the quantitative determination of Urea concentration in serum or EDTA plasma or Urine

INTENDED USE

The TRUEchemie Urea Test Kit (Berthelot - End Point) is used for the quantitative determination of Urea concentration in human serum or plasma or Urine.

INTRODUCTION

Elevated serum urea levels may be due to pre-renal or post-renal etiologies. Pre-renal causes could be cardiac related or due to increased protein catabolism. Renal causes include glomerulonephritis, chronic nephritis, nephrotic syndromes and other kidney diseases. Post-renal causes include obstruction of the urinary tract.
Urea kit incorporates liquid reagents for estimation of urea photometrically by the Berthelot method. This method offers a high degree of precision and specificity due to urease enzyme and high sensitivity due to high molar absorption of the final colour.

PRINCIPLE

Urease catalysis the conversion of Urea to ammonia and carbon-di-oxide. The ammonia released reacts with a mixture of salicylate, hypochlorite and nitroprusside to yield a bluegreen coloured compound (Indophenol). The intensity of colour produced is proportional to the concentration of urea in the sample and is measured photometrically at 578 nm or with yellow filter.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Salicylate} + \text{Hypochlorite} \xrightarrow{\text{Nitroprusside}} \text{2,2 -Dicarboxy Indophenol}$$

PACK SIZE

Kit size	**2 x 100 ml**
Cat. no.	**ADX242**
Kit contents	
1) Urease Reagent (R1)	1 vial
2) Buffer for Urease Reagent (R1A)	1 x 100 ml
3) Alkaline Hypochlorite Reagent (R2)	1 x 100 ml
4) Urea Standard (40 mg/dL)	1 x 5 ml

REAGENTS COMPOSITION

1) Urease working Reagent

Phosphate buffer pH 6.8	:	20.00 mmol/L
Sodium salicylate	:	61.00 mmol/L
EDTA-Na_2	:	1.34 mmol/L
Urease	:	< 23 U/mL
Stabilizers		

2) Alkaline Hypochlorite Reagent (R2)

Alkaline hypochlorite	:	70 mmol/L
NaOH	:	180 mmol/L

3) Urea Standard

Urea concentration	:	40 mg/dL

REAGENT PREPARATION

Urease working reagent
1. Transfer the entire Urease Reagent (R1) into Buffer for Urease Reagent (R1A) with the new microtip.
2. Once the Urease Reagent (R1) is transferred, rinse the Urease Reagent (R1) vial with little Buffer for urease reagent and transfer the residual enzyme to ensure better reconstitution.
3. The reconstituted reagent is stable for 4 months when proper storage conditions are strictly maintained.
4. Slight haziness /turbidity in the enzyme concentrate vial disappears once added to Urease reagent and does not affect test performance and results.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The reagent contains sodium hydroxide that is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.
5. The disposal of the residues has to be done as per local legal regulations.

CALIBRATION

The procedures are calibrated with the standard solution which is included with each series of tests. The absorbance is used to calculate the results.

REAGENT STORAGE & STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8 ºC and contaminations are prevented during their use. Do not use reagents over the expiration date.

SPECIMEN COLLECTION AND STORAGE

Serum, Plasma and urine.
Urea will remain stable in serum for at least 1 day at room temperature (≤ 25 ºC), 5 days at 2-8ºC and 6 months when frozen (-20 º C). In urine, urea will remain stable, when kept at 2-8ºC, for 5 days, provided that the pH value is lower than 4.
If a urine sample is to be assayed, it should be previously diluted 1/100 with deionized water. Multiply the final result by 100.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes.
2. Test tubes/rack
3. Timer
4. 37°C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 578 nm (570–620).

TEST PROCEDURE

Primary wavelength 578 nm
Temperature 37°C
Prewarm the reagent to reaction temperature.

	Blank (µL)	Standard (µL)	Sample (µL)
Urease working Reagent	1000	1000	1000
Urea Standard	--	10	--
Sample	--	--	10
Mix and incubate for 3 min at 37ºC or 5 min at room temperature (≤ 25ºC)			
Alkaline Hypochlorite Reagent (R2)	1000	1000	1000

Mix well and incubate for 5 min at 37ºC or 10 min at room temperature. After incubation, zero the Photometer with the reagent blank. Read and record the incubated standard and samples.
Final Colour stability: a maximum of 4 hours, when protected from direct sunlight.

(a) Serum / Plasma Urea in mg/dl

$$\text{Calculation: } \frac{\text{Sample OD}}{\text{Standard OD}} \times 40 = \text{mg Urea / dL}$$

(b) Blood Urea Nitrogen in mg/dl = a x 0.467

(c) Urine Urea in mg/24 hours = a x 24 hrs urine volume in litres

S.I. Units (mg/dl) x 0.1665 = mmol/L

QUALITY CONTROL

Quality Controls are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUE

Serum/plasma : 10 - 50 mg/dL
Urine : 25 - 43 g/ 24 hrs.
Serum/Plasma Urea Nitrogen : 5 - 23 mg/dL
It is strongly recommended that each laboratory establish its own normal range

PERFORMANCE CHARACTERISTICS

Sensitivity : 2.5 mg/dL
Linearity: Up to 400 mg/dL under the described assay conditions. If the concentration is greater than linearity (400 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION:

Intra-assay precision within run (n=10)	**Mean (mg/dL)**	**SD (mg/dL)**	**CV (%)**
Control Level -1	30.6	0.3	1.0
Control Level -2	101.0	0.5	0.5

Inter-assay precision run to run (n=12)	**Mean (mg/dL)**	**SD (mg/dL)**	**CV (%)**
Control Level -1	30.0	0.3	1.1
Control Level -2	99.6	0.5	0.5

The reagent was tested for 12 days, using two different Urea concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie Urea reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.996 and the regression equation is y=0.995x+0.228. The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Any glassware contamination by ammonium salts or ammonia should be avoided. Serum samples should be free from hemolysis and turbidity. Fluoride as well as ammonium heparinate inhibit the reaction.

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEM PARAMETERS

Mode	:	End point
Std. conc.	:	40
Wave length	:	578 nm
Units	:	mg/dL
Flow cell temp.	:	37°C
Blank	:	Reagent
Urease working Reagent	:	1000 µL
Sample volume	:	10 µL
Alk. Hypochlorite Reagent	:	1000 µL
Incubation	:	3 + 5 min. at 37°C
Low normal	:	10
High normal	:	50
Sensitivity	:	2.5
Linearity	:	400
Reaction Slope	:	Increasing

REFERENCES

1. Foster, L.B., Hochholzer, J.M. (1971), Clin. Chem., 17, 921-925. Wilcox, A., Wallace, E.C.,Sterling, R.E., David, H.A., Ware, A.G. (1966), Clin. Chem. 12, 151-157.
2. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use	Catalogue number	Caution
In vitro diagnostic medical device	Batch code	Non-sterile
Temperature limit 2-8 °C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep dry

Do not use if package is damaged

Keep away from sunlight

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