Urea

Reagent for quantitative In-vitro-determination of urea in blood

HST 321

Order No. **HST 321**
Contents: 20 tests
20 pipette tips, 500µL

Method
Berthelot modified
Determination in diluted plasma after separation from erythrocytes by centrifugation

Sample material
Capillary or EDTA blood (fresh).
Set capillary blood immediately in reaction tube "R".
Stability of the sample in reaction tube "R":
6 hours (+15°C to +25°C)

Reagents
Contents / concentrations:
1. Starter reagent (caps in PE-bottle)
   Dichloro-cyanuric acid sodium salt 3.4 mmol/L, sodium nitroprussid 2.9 mmol/L
2. Buffer solution (pre-portioned in round cuvettes)
   Tri-sodium citrate 118 mmol/L, Sodium salicylate 217 mmol/L, Sodium hydroxide 32 mmol/L
3. Enzymatic solution (Reaction tubes "R")
   Urease > 20 kU/L, Sodium chloride 9 g/L, sodium azide < 0.1 %

Safety information
The starting reagent contains sodium nitroprussid (< 0.5%). Harmful to health when swallowing. The buffer solution contains sodium hydroxide (< 0.2%). It irritates skin and eyes. When contact with eyes swill immediately with water. The enzymatic solution contains sodium azide (< 0.1%). Do not swallow and avoid contact with skin and mucous membranes.
A safety data sheet is available on request.[1)]

Storage and shelf life
The reagent have to be kept in a dark place at a temperature between + 2°C and + 8°C until the expiry date indicated on the packaging.

Measurement conditions

Measurement devices:	Diaglobal Photometer
Meas. wavelengths:	520 nm, 546 nm
Temperature:	Room temperature
Additionally required:	Mini centrifuge

Measurement range
10 - 100 mg/dL (1.7 – 16.7 mmol/L)
Should values exceed this range, repeat determination with 10 µL sample or dilute sample 1+1 with saline solution. Multiply the result by 2.

Tips
1. The test is disturbed by ammonia. Therefore smoking is prohibited during the determination.
2. The "R"-tubes in Mini-centrifuge needs to be equally distributed, see operating instructions.

Working instructions
A. Single measurement
- Withdraw 20µL capillary blood from finger pulp or earlobe and insert in reaction tube "R"
- Transfer blood in reagent solution by mixing strongly
- Insert reaction tube "R" in Mini centrifuge and centrifuge for 1 minute
- Pipette 500µL supernatant into round cuvette (blank cuvette)
- Select the <Urea> test.
- Set the photometer's zero point using the blank cuvette
- Remove blank cuvette
- Screw the cap from PE-bottle onto the blank cuvette, dissolve the starter reagent by mixing very strongly (analysis cuvette)
- Press [ON/ENTER] and insert immediately analysis cuvette into photometer
- Wait 10 minutes for result

B. Measurement of series (up to 20 samples)
- Select the <Urea> test.
- Insert the blank cuvettes one after another and measure the zero points.
- Screw the caps from PE-bottle onto all blank cuvettes, dissolve the starter reagents by mixing all cuvettes very strongly at the same time (analysis cuvettes)
- Press [ON/ENTER], insert the first analysis cuvette immediately and wait 10 minutes for result of sample 01.
- Insert the other analysis cuvettes one after another in the same order as of the blank measurement. The result of the respective analysis cuvette can be read immediately.

Calculation
The method is calibrated with whole blood. The calculation formula is programmed in the Diaglobal Photometers. If human or control sera are used (or ring test samples resp.), multiply the indicated value at measurements with DP 300 by 0.75 and at measurements with DP 310 by 0.73.

Quality assurance
For quality assurance we recommend universal control sera from company Roche, www.roche.de:
PreciControl ClinChem Multi 1 / Multi 2 (4 x 5 mL)
Order-No.: 05 947 626 190 / 05 947 774 190
Ref.: Roche / Hitachi analyzers, Method: kinetic UV

Reference values[2)]

Serum, plasma	mg/dL	mmol/L
Men < 50 years	19 - 44	3.2 - 7.3
Men > 50 years	18 - 55	3.0 - 9.2
Women < 50 years	15 - 40	2.8 - 7.2
Women > 50 years	21 - 43	2.6 - 6.7
Children up to 13 years	11 - 36	1.8 - 6.0
Children up to 19 years	18 - 45	2.9 - 7.5

Summary
Urea is the end product of protein and amino acid decomposition, which takes place in the liver. Excretion occurs through glomerular filtration over the kidneys. The urea concentration in serum depends on the renal function, the renal circulation, the uric volume, and the protein supply through nourishment. If the glomerular filtration rate is highly derogated and the protein absorption exceeds a value of 200 g/day, elevated urea counts will arise with affected kidneys.

Indications / diagnostic significance[2,3)]
- Diagnosis and devolution control of renal insufficiency
- Control of diet with conservative therapy of chronic renal insufficiency
- Differential diagnosis on comatose status

In the sports sector[4)], the serum urea level increases during longtime activity since the muscle cells increasingly supply their energy requirement from the amino acid decomposition after clearance of the glycogen storages. Therefore, the determination of the serum urea level allows control of stress (regeneration) during training and after tournaments.

Nowadays, for the determination of urea only enzymatic methods are applied that are based on the cleavage of urea by means of urease. The Diaglobal colorimetric test HST 321 was developed especially for onlocation analyses and allows a direct determination of the urea concentration in plasma. Capillary or venous blood is used as sample material.

Measurement principle
The sample is diluted with saline solution containing urease and centrifugated shortly. Here, the erythrocytes become separated. At the same time, cleavage of urea takes place. The emerging NH_3 is converted into a green dye by means of salicylate and a chlorination substance (modified Berthelot reaction[5)]), which can be measured at 546 or 520 nm.

$$\text{Urea} + H_2O + 2H^+ \xrightleftharpoons{\text{Urease}} 2\,NH_4^+ + CO_2$$

$$NH_4^+ + \text{Salicylate} + \text{Dichloroisocyanurate} \rightarrow \text{Indamine dye}$$

Performance parameters
Specificity / interferences[6)]
No interferences due to ascorbic acid, glucose, and other reducing substances. Values are independent of haematocrit. Interferences due to pharmaceuticals are not known.

Inaccuracy
The reproducibility was checked using human and control samples.

In series [n = 20]	Average [mg/dL]	Standard deviation [mg/dL]	VK [%]
Sample 1 Sample 2	19.0 138	1.01 4.41	5.3 3.2
From day to day* [n = 20]	**Average [mg/dL]**	**Standard deviation [mg/dL]**	**VK [%]**
Sample 1	56.0	1.46	2.6

* Control sample

Analytic sensitiveness
Lower detection limit: 5 mg/dL (0.8 mmol/L)

Comparison of methods
A comparison of the Diaglobal test HST 321 (y) and the Diaglobal UV test HST 013 (x) resulted in the following correlation according to the Passing/Bablok[7)] process:

$$y = 1.030x - 0.678$$
$$r = 0.997$$

n = 80
Cconcentration range: 19 - 200 mg/dL

Bibliography

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