METHOD SPESIFICATION
Faculty of Biosciences, NMBU

Method name: Urea
BIOVIT-nr.: Msp1012

1. Method of analysis / Principle / Main instrument:

Urea is mainly analyzed in serum, plasma, urine, and milk. Urea is a product of protein metabolism and is excreted by the kidneys. The level of urea in blood is directly related to the protein metabolism and the endogenous nitrogen catabolism which mainly occurs in the liver. It is inversely proportional to the level or urine excretion. It is a kinetic, enzymatic UV method.

Reaction:

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + CO_3^{2-}$$

$$NH_4^+ + \alpha\text{-ketoglutarate} + \text{NADH} \xrightarrow{\text{glutamate dehydrogenase}} \text{L- glutamate} + \text{NAD} + H_2O$$

The increase in NADH concentration is directly related to the urea concentration and is measured photometrically.

Main instrument: RX Daytona + (Randox Laboratories Ltd, UK). 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom).

Kit: Urea (UR8334). Reportable range: 0.50-62.0 mmol/L.

2. History - instrument transitions and method modifications:

At IHA, the method has been modified to also measure urea in milk. The milk must be centrifuged, and the sample must be taken from under the cream layer.

Instrument transition 1995: from Encore to Cobas Mira S spectrophotometer (March-1995)

- Modified 11.01.00 after transition from Roche to ABX reagents.
- Modified 12.02.04 after transition from ABX reagents to Pentra reagents.

Instrument transition 2010: from Cobas Mira to MaxMat (August-2010)

- Method modification after transition to MaxMat spectrophotometer with reagents, controls and standard from ILS Laboratories ScandinaviaAS.

The reference is taken from the reagent attachment:

➢ Method Comparison and Bias Estimation Using Patient Samples, Approved Guideline, 2nd ed., NCCLS document EP9-A2, Vol. 22, No. 19, 2002.

Instrument transition 2018: from MaxMat to RX Daytona + (October-2018)

➢ Method modification after transition to RX Daytona + with reagents, controls, and calibrators from Randox Laboratories Ltd, United Kingdom.

3. Requirements for the degree of grinding and temperature of the sample for storage before analysis.

Blood samples for serum are taken into a vacutainer ready to use, without additives (read cap). Blood samples are taken into vacutainer ready to use (with addition av heparin, EDTA, eller iodacetat. -heparin (NOT ammonium heparin).
Do not use fluoride as it inhibits urease! Approximately 0,5-1mL of plasma or serum is needed for the analysis. The samples are stabile for 24 h at room temperature or for 1 week at 4 °C (or 2-3mnd. at/ -15-20 °C).
The urine samples have been centrifuged and diluted with distillated water in a ratio of 1:20 to 1:50 prior to analysis.
About 5 mL of urine is needed. The urine sample is stable up to 4 days at 4-8 ° C. By adding Thymol or lowering the pH below 4, the bacterial activity in the urine sample is stopped.
Centrifuge the milk. Sample amount: Approx. 3-5mL.
The samples can be frozen at /-20 °C i 2-3m.

4. Contact person:

Lab leader: Hanne Kolsrud Hustoft
Responsible for analysis: Milena Bjelanovic / Elin Follaug Johnsen

5. Other literature:

The references are taken from the reagent attachment:

-Newman, D. J., Price C. P., Non protein Nitrogen Metabolite. Tietz Fundamentals of Clinical Chemistry, 5ème Ed., Burtis, C. A. & Ashwood. E. R. (W. B. Saunders eds. Philadelphia USA), (2001), 414.
-Tietz,N.W., Clinical guide to laboratory tests, 3e`me Ed., (W.B. Saunders eds.Philadelphia USA), (1995), 622.
- First, M. R, Renal function. Clinical Chemistry: Theory, Analysis, Correlation, 4ème Ed.,Kaplan, L. A, Pesce, A. J., Kazmierczak, S. C.,(Mosby Inc. Eds St Louis USA), (2003), 477 et appendice.
- Bretaudière, J. P., et al., Direct Enzymatic Determination of Urea in Plasma and Urine with a Centrifugal Analyzer. Clin. Chem., (1976), 22, 1614.
-Fawcett, J.K., Scott, J.E., A Rapid and Precise Method for the Determination of Urea.

J.Clin.Path., (1960), 13,156.

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