UREA UV FL

AZ F080 CH	4 x 20 ml
AZ F245 CH	12 x 20 ml
AZ F400 CH	8 x 50 ml
AZ F600 CH	5 x 120 ml
AZ 100F CH	5 x 200 ml

SUMMARY OF TEST

Urea is the major nitrogen-containing metabolic product of protein catabolism in humans. The biosynthesis of urea from amino nitrogen-derived ammonia is carried out exclusively by hepatic enzymes of the urea cycle. More than 90% of urea is excreted through the kidneys, with losses through the gastro-intestinal tract and skin accounting for most of the remaining minor fraction. Urea is neither actively reabsorbed nor secreted by the tubules but is filtered freely by the glomeruli. More importantly, urea production is too dependent on several non-renal variables such as diet and hepatic synthesis to make it useful as a measure of GFR. At present, utility of urine urea measurement is limited to partition of urinary nonprotein nitrogenous compounds among the various nitrogen-containing constituents and to the measurement of the urea production rate. In chronic renal failure, the osmotic diuresis in the remaining functional nephrons limits the back-diffusion of urea so that urea clearance approaches inulin clearance. Measurement of plasma or serum urea, however, may provide useful clinical metabolic information in particular circumstances.
A wide variety of renal diseases can cause an increase in plasma urea concentration. Mild dehydration, high-protein diet, the increased protein catabolism, muscle wasting as in starvation, reabsorption of blood proteins after a gastrointestinal hemorrhage, treatment with cortisol or its synthetic analogues, and decreased perfusion of the kidneys may cause an azotemia that is called prerenal azotemia. Impaired perfusion may be due to decreased cardiac output or shock secondary to blood loss or other causes. The key to identifying the azotemia as prerenal is documentation of an increase of plasma urea without concomitant increase of plasma creatinine. Post renal azotemia is caused by conditions that obstruct urine outflow through the ureters, bladder, or urethra; examples of these conditions are nephrolithiasis, prostatism, and tumors of the genitourinary tract. With obstruction, both plasma urea and creatinine increase, but there is a disproportionately greater rise of urea than of creatinine because the obstruction of urine flow causes backpressure on the tubule and back-diffusion of urea into the blood from the tubule.
Methods for the determination of urea could be indirect methods, based on preliminary hydrolysis of urea with urease, followed by some process that quantitates the ammonium ion. Spectrophotometric approaches to ammonium quantitation include the Berthelot ‘s reaction and the enzymatic assay with glutamate dehydrogenase. For serum or plasma assays, the reaction system is usually formulated with urease so that the addition of sample containing urea starts the reaction. Decrease in absorbance, resulting from the glutamate dehydrogenase reaction, is monitored at 340 nm in either an equilibrium or kinetic mode. The coupled-enzyme system has been automated on several analytical systems.

PRINCIPLE OF THE METHOD

The urease hydrolizes urea in sample to release ammonium ions, which react with 2-oxoglutarate and NADH in presence of glutamate dehydrogenase to form glutamate and NAD^+. The decrease of absorbance is measured at 340 nm.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

Reagent A **F080: 4 x 16 ml (liquid) blue cap**
F245: 12 x 16 ml (liquid) blue cap
F400: 8 x 40 ml (liquid) blue cap
F600: 4 x 120 ml (liquid) blue cap
100F: 4 x 200 ml (liquid) blue cap

Reagent B **F080: 1 x 16 ml (liquid) red cap**
F245: 2 x 24 ml (liquid) red cap
F400: 2 x 40 ml (liquid) red cap
F600: 1 x 120 ml (liquid) red cap
100F: 1 x 200 ml (liquid) red cap

Composition in the test: CAPSO buffer 8 mM pH 7.60, 2-Oxoglutarate 7.5 mM, Urease > 8 KU/l, GLDH > 800 U/l, NADH 0.25 mM, stabilizers.

Standard: urea 50 mg/dl - 5 ml
Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Serum as starter procedure:
Codes F080/F245: add 4 ml of reagent B to a bottle of reagent A.
Code F400: add 10 ml of reagent B to a bottle of reagent A.
Code F600/100F: mix 1 part of reagent B with 4 parts of reagent A.
Stability of working reagent: preferably within 60 days at 2-8°C, away from light sources.

Reagent as starter procedure:
use separate reagents ready to use.
Stability: up to expiration date on labels at 2-8°C;
Stability since first opening of vials: preferably within 60 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general “Good Laboratory Practice” (GLP) guidelines.

SPECIMEN

Serum, plasma (avoid ammonium heparinate). Urine.
Urea is stable 3 days at 2-8°C.
Dilute urine sample 1:100 with deionized water.

TEST PROCEDURE (sample as starter)

Wavelenght: 340 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	blank	standard	sample
working reagent	2 ml	2 ml	2 ml
incubate at 37°C for 5 minutes			
water	20 µl	-	-
standard	-	20 µl	-
sample	-	-	20 µl

Mix, incubate 30 seconds at 37°C, then record absorbance as A_1. After exactly 60 seconds, record again absorbance as A_2.

TEST PROCEDURE (reagent as starter)

Wavelenght: 340 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	blank	standard	sample
reagent A	2 ml	2 ml	2 ml
water	25 µl	-	-
standard	-	25 µl	-
sample	-	-	25 µl
incubate at 37°C for 5 minutes			
reagent B	500 µl	500 µl	500 µl

Mix, incubate 30 seconds at 37°C, then record absorbance as A_1. After exactly 60 seconds, record again absorbance as A_2.

RESULTS CALCULATION

Serum/plasma sample:

$$\text{urea mg/dl} = \frac{A_2\text{-}A_1 \text{ (sample)}}{A_2\text{-}A_1 \text{ (standard)}} \times 50 \text{ (standard value)}$$

Random urine sample:

$$\text{urea mg/dl} = \frac{A_2\text{-}A_1 \text{ (sample)}}{A_2\text{-}A_1 \text{ (standard)}} \times 50 \times 100$$

(standard value and dilution)

24 hours urine sample (urea g/24h):

$$\frac{[A_2\text{-}A_1 \text{ (sample)}] / [A_2\text{-}A_1 \text{ (standard)}] \times 50 \times 100 \times \text{urine volume}}{1000}$$

(standard value, dilution factor and diuresis in decilitres)

EXPECTED VALUES

Adults:	10 - 50 mg/dl	(1.7 - 8.3 mmol/l)
Urine:	20 - 35 g/24h	(332 - 580 mmol/24h)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:
QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values
QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
the method is linear up to 300 mg/dl.
If the value is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
the limit of detection is 1 mg/dl.

Interferences
no interference was observed by the presence of:

hemoglobin	≤ 500 mg/dl
bilirubin	≤ 44 mg/dl
lipids	≤ 600 mg/dl

Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	46.19	0.65	1.40
sample 2	140.89	2.72	1.90

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	42.77	1.91	4.50
sample 2	144.29	6.72	4.70

Methods comparison
a comparison between Chema and a commercially available product gave the following results:

Urea UV FL Chema = x
Urea competitor = y
n = 100

y = 0.9746x + 3.03 mg/dl $r^2 = 0.986$

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. Refer to special instructions/safety data sheets.

REFERENCES

Falke, H.N. Schubert, G.E.Klin.Wschr.42 (1965)
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).
HU Bergmeyer - Methods of enzymatic analysis, (1987).

MANUFACTURER

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SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions

IUS-7.5 UK rev. 23/05/2011