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 F500 CH	5 x 100 ml
AZ F600 CH	5 x 120 ml
AZ 100F CH	5 x 200 ml

INTENDED USE

Reagent for quantitative in vitro determination of urea in biological fluids.

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 gastrointestinal 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 nonrenal variables such as diet and hepatic synthesis to make it useful as a measure of GFR.

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.

UREA R1 **F080: 4 x 16 ml (liquid) blue cap**
F245: 12 x 16 ml (liquid) blue cap
F400: 8 x 40 ml (liquid) blue cap
F500: 4 x 100 ml (liquid) blue cap
F600: 4 x 120 ml (liquid) blue cap
100F: 4 x 200 ml (liquid) blue cap

UREA R2 **F080: 1 x 16 ml (liquid) red cap**
F245: 3 x 16 ml (liquid) red cap
F400: 2 x 40 ml (liquid) red cap
F500: 1 x 100 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 in code AZ F080 CH: 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 R2 to a bottle of reagent R1.
Code F400: add 10 ml of reagent R2 to a bottle of reagent R1.
Code F500/F600/100F: mix 1 part of reagent R2 with 4 parts of reagent R1.
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

UREA R1: It is not classified as hazardous.

UREA R2: Warning. Causes serious eye irritation (H319). Causes skin irritation (H315). Wear protective gloves. Eye protection (P280). IF ON SKIN: Wash with plenty of water (P302+P352). IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing (P305+P351+P338). If eye irritation persists: get medical advice (P337+P313).

Standard: It is not classified as hazardous.

SPECIMEN

Serum, plasma heparin ammonium. 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 R1	2 ml	2 ml	2 ml
water	25 µl	-	-
standard	-	25 µl	-
sample	-	-	25 µl
incubate at 37°C for 5 minutes			
reagent R2	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:

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

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:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

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.
P501: Dispose of contents according to national/international regulations.

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	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use-by date
	caution
	consult instructions for use

CE