Urea – Instructions for use (IFU)

Manufactured exclusively for: ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

R1: 4 x 50 mL – R2: 4 x 13,5 mL • REF A-R0200000901

R1: 2 x 16,7 mL – R2: 1 x 8,5 mL • REF R3330000026

IVD CE

INTENDED USE
Product for use in the quantitative determination in vitro of the Urea concentration in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

CLINICAL SIGNIFICANCE
Urea is the product of metabolism with the highest nitrogen content deriving from protein catabolism in the human species and represents over 75% of protein nitrogen. Over 90% of Urea is secreted by the kidneys, while the gastrointestinal tract and the skin account for most of the remaining fraction. There are three factors which determine the plasma urea concentration: renal perfusion and amount of water secreted, rate of urea synthesis, and rate of glomerular filtration. Numerous increases in the plasmatic urea concentration are caused by a wide range of renal diseases. In children, protein catabolism is reduced due to the greater protein requirements necessary for growth, therefore the serum urea concentrations are lower compared to adults. Pregnant women show urea levels outside the normal range due to the greater protein requirements of the fetus, in addition to the renal hyperperfusion.

PRINCIPLE
In the presence of urease, urea is hydrolyzed into ammonium ions and carbon dioxide. In the presence of glutamate-dehydrogenase (GLDH), the ammonium ion produced reacts with α-ketoglutarate (α-KG) and NADH to form glutamate and NAD. The consumption of NADH over a given period, determined at λ 340 nm, is proportional to the Urea concentration in the test sample.

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

$$NH_3 + \alpha - KG + NADH \xrightarrow{GLDH} L - Glutamate + NAD$$

REAGENTS
A-R0000000021 - R1: 4 x 50 mL - R2: 4 x 13,5 mL
Reagent 1: n° 4 vials x 50,0 mL ready to use
Reagent 2: n° 4 vials x 13,5 mL ready to use
R3330000026 - R1: 2 x 16,7 mL - R2: 1 x 8,5 mL
Reagent 1: n° 2 vials x 16,7 mL ready to use
Reagent 2: n° 1 vials x 8,5 mL ready to use

Concentrations

Reagent 1:		
	Conc.	**U.M.**
Good's Buffer (pH 7.6)	110	mM
Adenosine-diphosphate (ADP)	1.10	mM
Urease	≥ 7500	U/L
Glutamate-dehydrogenase (GLDH)	≥ 1200	U/L
Reagent 2:		
Good's Buffer (pH 10.2)	104	mM
α-ketoglutarate (α-KG)	68	mM
NADH	1.22	mM

Precautions
Kit for professional laboratory use, used only by qualified and properly trained technical personnel, under the supervision of a doctor in charge of the laboratory. In addition to any risk statements relating to active components, reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in EC 1272/2008 Regulation and subsequent amendments and additions. However, it is recommended to handle the reagents according to the rules of good laboratory practice.

Reports of serious incidents
In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

Storage and stability
Store at 2-8°C; protect from direct light. When correctly stored, the reagent is stable up to the expiry date reported on the label. A slight variation in the composition of the components may occur between batches, but this has no effect on the test results.
After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protected from contamination, evaporation, direct light and stored at correct temperature.

SAMPLE COLLECTION
Type of sample and storage
Serum or heparinised plasma samples should be used. Do not use anticoagulants containing fluorides. Urine samples collected in 24 hours, must be diluted 1:20.
Samples can be kept for 3 days at 4 - 8°C or for 6 months at - 20°C.

Precautions
All human samples must be handled and disposed of as potentially infectious materials.

Procedure
Quality control
Control sera with known titer of urea are commercially available for quality control, including certificates of analysis showing values and confidence limits. Normal and pathological control sera are available as "Normal control serum" cod. R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:

- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation
Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".
All applications that are not explicitly approved cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user.

Calibration
For calibration use the kit "Multicalibrator" code R0300000006. For ISE srl instruments, calibration is recommended every 10 days.

Traceability:
The value of Urea is visible in the insert of the packaging of the calibration serum.

Calibration Stability

Reaction conditions

Wavelenght (primary):	340 nm
Wavelength (secondary):	620 nm
Temperature:	37°C

Technique – Procedure with Reagent B as starter
Bring the reagents to the reaction temperature.

	U.M.	**Calib. Serum**	**Sample**	**Blank**
Reagent A	µL	1000	1000	1000
Calib. Serum	µL	10	-	-
Sample	µL	-	10	-
Water	µL	-	-	10
Mix gently and incubate at 37°C for 1-5 min. and add				
Reagente B	µL	250	250	250

Mix well and incubate at 37°C. Then read the first extinction value after 30 seconds from the addition of the sample, make the second reading after 60 seconds.
Read the absorbances of the sample and the calibration serum by subtracting the absorbance of the reagent white.
The reaction volumes can be varied proportionately, the calculation remaining unchanged.

Results

$$\frac{\Delta \text{ D. O. Sample}}{\Delta \text{ D.O. Calib. Serum}} \times \text{Calibration Concentration} = \text{mg/dL di Urea}$$

Reagents included in the kit
The reagents are described above.

Materials required but not supplied in the kit
Calibrators and controls.

Sclavo Diagnostics International S.p.A
Via Po 26-28 – Loc. Pian dei Mori – 53018 Sovicille (SI) (Italy)
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NORMAL VALUES
Serum or plasma:
- Urea: 10 - 50 mg/dL (1.66 – 8.30 mmol/l)
- Uric nitrogen: 6 - 20 mg/dL (0.99 – 3.32 mmol/l)

Urine:
- Urea: 26 – 43 g/24 h
- Uric nitrogen: 12 – 20 g/24 h

Each laboratory must establish its own normal values based on its local population.

ANALYTICAL CHARACTERISTICS/PERFORMANCE

Linearity
The method is linear up to 145 mg/dL of Urea. In the case of higher concentrations, repeat the test on samples diluted in physiological saline and multiply the result by the dilution factor.

Specificity
The method is specific for the determination of Urea, in the analytical conditions described.

Accuracy – Recovery
The recovery of Urea added to normal samples at known concentrations showed a result of 90.8%.

Interferences
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 20 mg/dL does not interfere in the reaction, haemoglobin interferes at concentrations above 50 g/L. For other interfering substances, refer to the bibliography.

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	90.9	2.26	2.48%	20
High	mg/dL	150	9.79	6.52%	20
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	90.9	5.96	6.55%	20
High	mg/dL	150	7.80	5.19%	20

Sensitivity
At λ 340 nm, the sensitivity in terms of detection limit is 7.61 mg/dL.

Comparative method
The Urea method (y) was compared with a similar commercial method.
A regression curve was determined: y = 1.0276x + 4.00; r = 0.9917; Samples tested = no. 100.

Disposal of reagents
Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

Manufacturer:
Sclavo Diagnostics International
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Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

Reference

1. **Talke H, Schubert GE**: Klin Wchers., (1965), 42,174.
2. **Kaplan LA, Pesce AJ**:"Clinical Chemistry", Mosby Ed. (1996)

REVISION	DATE	CHANGE
Rev.C	01/2024	Edit header and "Reagents" paragraph.