Urea UV – method Enzymatic (Urease)
Instruction for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB NDIKO	REF B75182536	n° 10 vials x 48 mL (R.A) n° 10 vials x 12 mL (R.B)
	REF B75182537	n° 12 vials x 16 mL (R.A) n° 12 vials x 4 mL (R.B)
CHEMILAB	REF B81180171	n° 2 vials x 32 mL (R.A) n° 2 vials x 8 mL (R.B)
	REF B81180172	n° 8 vials x 32 mL (R.A) n° 8 vials x 8 mL (R.B)

INTENDED USE
Product for use in the quantitative determination in vitro of the concentration of the Urea 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.

PRINCIPLE OF THE METHOD
Method Enzymatic (Urease) UV. 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 of time, determined at λ 340 nm, is proportional to the Urea concentration in the test sample.

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

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

Storage and stability
= storage temperature 2-8°C
stored at 2-8 ° C avoiding direct light, the reagents are stable until the expiration date printed on the label.

Concentrations

Reagent A:		
	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 B:		
Good's Buffer (pH 10.2)	104	mM
α-ketoglutarate (α-KG)	68	mM
NADH	1.22	mM

Reagents included in the kit
The reagent is described above.

Materials required but not supplied in the kit
Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS
1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles)
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting 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.

PROCEDURE
Quality control
Control sera with a known titer of Urea are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration
For calibration use the "Calibrator serum Sclavo" Code B35181702

Traceability
The Urea traceability is reported in the package insert supplied with the calibrator serum.

SAMPLE
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.
Urine samples collected within 24 hours should be diluted 1:20.

REAGENT PREPARATION
Add 1 volume of Reagent vial B to 4 volumes of Reagent A, and mix gently. The reagent is then ready for use. Working solutions stability is 30 days at 2 - 8°C. A slight variation in the coloration from lot to lot, does not affect test results.

Automation
The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD
The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions
Wavelength (primary): 340 nm
Temperature: 37°C
Reaction End-point (increasing reaction)

Technical – procedure with Serum as starter
Bring the reagents to reaction temperature and operate away from direct light.

	U.M.	Blank	Calibrator Serum	Sample
Reagent	µL	1000	1000	1000
Blank	µL	10	-	-
Calibrator Serum	µL	-	10	-
Sample	µL	-	-	10

Mix, then incubate at 37°C. Measure the absorbance values of first reading after 30 seconds for a sample adding, read a second time after 60 seconds.
The reaction volumes can be altered proportionately without any change in the calculation.

Results:
Manual Method
Calculation of Urea concentration:

$$\frac{\Delta D.O.\ Sample}{\Delta D.O.\ Calibrator Serum} \times Conc. Calibrator Serum = Urea\ mg/dL$$

Automation
The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

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IVD CE

REFERENCE RANGE

Serum or plasma:

- Urea: 10 - 50 mg/dL
- Uric nitrogen: 6 - 20 mg/dL

Urine:

- Urea: 26 – 43 g/24 h
- Uric nitrogen: 12 – 20 g/24 h
- Each laboratory should calculate its own normal values on the basis of its local population.

ANALYTICAL CHARACTERISTICS/PERFORMANCE

Linearity

The method is linear up to 300 mg/dL (49.95 mmol/L). If the value in the sample exceeds the linearity limit of the method, dilute the sample with saline and multiply the result for the dilution factor.

Trueness

The Trueness of the analytical results has been determined accordingly to the CLSI guidelines, using commercial control sera. The data obtained are shown in the following table.

Serum – Plasma

Level	Replicates	Mean	SD	CV%	Recovery
Low	5	31.2	0.447	1.43	95,9 %
High	5	139	1.225	0.88	93,9 %

Urine

Level	Replicates	Mean	SD	CV%	Recovery
Low	25	95.28	3.87	4.1	105,8 %
High	25	179.73	5.86	3.3	105,7 %

Interferences

Interference	Limits
Bilirubin	**20 mg/dL**
Hemoglobin	**5000 mg/dL**

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	20.3	0.76	3.73	30
High	mg/dL	136	2.99	2.19	30
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	24.1	0.89	3.71	30
High	mg/dL	140	3.60	2.59	30

Limit of Sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 5.0 mg/dL (0.83 mmol/L) of urea.

Comparison between methods

The method was compared with a similar commercially available method, analyzing 36 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-1.218
Slope	1.02126
Correlation Coeff. (R)	0.999

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	

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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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