Creatinine – Colorimetric Enzymatic Method

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182580	n° 4 vials x 30 mL (R.A) n°4 vials x 10 mL (R.B)
	REF B75182581	n° 4 vials x 14 mL (R.A) n°4 vials x 5 mL (R.B)
CHEMILAB	REF B81180101	n° 2 vial x 12 mL (R.A) n° 1 vials x 8 mL (R.B)

INTENDED USE

Quantitative in vitro determination of the concentration of Creatinine in human serum, plasma and urine. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

Creatinine is synthesized in the kidneys, liver and pancreas and is successively transported in blood to other organs including muscles and the brain. Since the quantity of endogenous Creatinine produced is proportionate to muscle mass, production varies based on age and sex. The influence of the amount of meat in a subject's diet is estimated at around 10%. However, overall, daily fluctuation in Creatinine levels in the diet causes only minor variation in daily excretion. Elevated creatinine levels are seen in cases of acute and chronic kidney deficiency and dehydration.

PRINCIPLE OF THE METHOD

The method is based on colorimetric enzymatic determination of creatinine eliminating many of the interferences identified with the Jaffè method. The potential interference of endogenous creatinine and sarcosine is eliminated by the reaction of creatine amidohydrolase, sarcosine oxidase and catalase before creatinine is determined in the final reaction where absorption is increased to 550 nm. Ascorbate oxidase is included in the reagent to eliminate the influence of ascorbate in the sample.

$$\text{Creatinine} + H_2O \xrightarrow{CRN} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{CR} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{SOX} \text{Formaldehyde} + \text{Glycina} + H_2O_2$$

$$2H_2O_2 + 4\text{-Aminoantipyrina} + \text{EHSPT} \xrightarrow{POD} 4H_2O + \text{Quinone Colorant}$$

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.**
MOPS	25	mmol/L
N-etil-n-sulfopropile-m-toluidine (TOPS)	0.5	mmol/L
Creatinase (CR)	10	U/mL
Sarcosina Ossidasi (SOX)	5	U/mL
Catalase	3	U/mL
EDTA	1	mmol/L
Reagent B		
MOPS	90	mmol/L
Peroxidase (POD)	5	U/mL
Creatininase (CRN)	30	U/mL
4-Aminoantipyrine	5,9	mmol/L
Sodium Azide	0,5	g/L

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 Creatinine are commercially available for quality control, with values and confidence limits included. . Normal and pathological control sera Sclavo Diagnostics 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 Creatinine traceability is reported in the package insert supplied with the "Calibrator Serum".

SAMPLE

Type of sample and storage

Heparinized plasma or serum samples should be used. Do not use anticoagulants containing citrates, oxalates or EDTA as these tend to remove the calcium through formation of complexes. The samples can be stored for 7 days at 20-25°C, 3 weeks at 4-8°C, 8 months at – 20°C.

PREPARATION OF THE REAGENT

Reagents are ready-to-use liquids. After opening, the reagents are stable until their expiry date if maintained under the conditions indicated in "Storage and stability". Their slight variation in coloring, from batch to batch, does not affect the 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\2r\/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):	546 nm
Temperature:	37°C
Reaction	End point (increasing reaction)

Technical – procedure with Reagent B as starter

Bring the reagents to reaction temperature and operate away from direct light.

	U.M.	Blank	Calibr. Serum	Sample
Reagent A	µL	1000	1000	1000
Calibr. serum	µL	-	30	-
Camp. Serum/Plasma	µL	-	-	30
Mix well and incubate at 37°C for 5 min. and add				
Reagent B	µL	350	350	350

Mix well and after 5 minutes of waiting take the reading at 37° C. Read the absorbances of the sample and the calibration serum subtracting the absorbance of the white reagent, complete the readings within 5 minutes.

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	U.M.	Blank	Calibr. Serum	Sample
Reagent A	µL	1000	1000	1000
Calibr. serum	µL	-	30	-
Camp. Serum/Plasma	µL	-	-	15
Water	µL	30	-	-
Mix well and incubate at 37°C for 5 min. and add				
Reagent B	µL	350	350	350

Mix well and after 5 minutes of waiting take the reading at 37 ° C. Read the absorbances of the sample and the calibration serum by subtracting the absorbance of the reagent white, complete the readings within 5 minutes The reaction volumes can be varied proportionally, the calculation remains unchanged

Reaction volumes can be varied proportionately, without altering the results

Results:

Manual Method

Calculatin of Creatinina:

$$\frac{\Delta \text{ . Sample O.D.}}{\Delta \text{ Calibration Serum O.D.}} \text{ x Conc. calibration serum (mg/dL)} = \text{Conc. Creatinine (mg/dL)}$$

Automation

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

REFERENCE RANGE

Serum

Woman mg/dL 0,3 – 0,8

mg/dL 0.4 – 1.1

Urine

Man/Woman mg/day 1000 – 1700

Each laboratory should establish its own normal values according to the population in which it operates.

ANALYTICAL CHARACTERISTICS/PERFORMANCE

Linearity

The reaction is linear up to 200 mg/dL.If the value exceeds the linearity limit of the method, dilute the sample and multiply the result by the dilution factor.

Accuracy

Commercial control sera were analyzed with the kit in question following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Level	Replicated	Mean (mg/dL)	DS	CV%	Recovery
Low	5	0,42	0,04	9,52	73,6 %
High	5	5,10	0,00	0,00	75,9%

Interferences

Interferente	Limite
Asorbic acid	**100 mg/dL**
Bilirubin	**40 mg/dL**
Haemoglobin	**500 mg//dL**
Urea	**300 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	1,60	0,016	1,0	40
High	mg/dL	4,68	0,043	0,9	40
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	1,60	0,054	3,4	40
High	mg/dL	4,68	0,116	2,5	40

Limit of Sensitivity

The limit of sensitivity was measured by analyzing scalar dilutions of a concentrated serum. Under the conditions established for this test the lowest detectable concentration is approximately 0.4 mg/dL creatinine.

Comparison between methods

The method was compared with another commercially available method, analyzing 50 human sera. The correlation data between the two methods are shown in the table below.

Parameter	Estimation
Intercept	-0,06
Slope	1,001
Correlation Coeff. (R)	0,99

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	

REFERENCES

1. **Crocker H., Shephard MDS., White GH** (1988) Evaluation of an enzymatic method for determining creatinine in plasma. J Clin Pathol 41: 576-581
2. **Badiou S, Dupuy AM, Descomps B, Cristolead, JP**. (2003) Comparison between the enzymatic vitros assay for creatinine determination and three other methods adapted on the Olympus analyzer, Journal of Clinical Laboratory Analysis: 17, 235 – 240
3. **Moss GA, Bondar RJL, Buzzelli DM**. (1975) Kinetic Enzymatic Method for determining serum creatinine. Clin Chem 21/10: 1422-1428
4. **Clinical Laboratory Standards Institute (CLSI).** User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition. EP15-A2.
5. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2.
6. **Clinical Laboratory Standards Institute (CLSI).** Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. EP09-A3.
7. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17.
8. **Clinical Laboratory Standards Institute (CLSI).** Interference Testing in Clinical Chemistry, – Third Edition. - EP07.
9. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition - EP06.

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

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