Calcium-A III – Method Arsenazo III

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182559	n° 6 vials x 60 mL
	REF B75182560	n° 8 vials x 20 mL
CHEMILAB	REF B81180061	n° 3 vials x 26 mL
	REF B81180062	n° 5 vials x 31 mL

INTENDED USE

Product for use in the quantitative determination in vitro of the Calcium 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

Calcium is one of the mineral elements present in the blood, and one of the constituents of bone tissue. It is present in small amounts in soft tissues and in extracellular fluids. About 50% of the calcium present in the blood is free, 40% is bound to proteins and 10% is present in compounds. About 80% of calcium bound to proteins is associated with albumin and the remaining 20% with globulins. Extracellular calcium has the role of maintaining intracellular calcium, mineralizing bone, blood coagulation and maintenance of the cell membrane.

PRINCIPLE OF THE METHOD

Method Arsenazo III. Calcium ions form a highly coloured complex with Arsenazo III at neutral pH. When read at λ 660 nm, the colour which has formed will correspond to the amount of calcium present in the sample.

Calcium + 2 Arsenazo III → Ca-Arsenazo Complex

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:			
	Conc.	U.M.	
Imidazol buffer (pH 6.75)	100	mmol/L	* GHS08
Arsenazo III	0.20	mmol/L	

Signal word **DANGER**

Contains: Imidazole (CAS 288-32-4)

H360D- May damage the unborn child.

P201 - Obtain special instructions before use.

P202 - Do not handle until all safety precautions have been read and understood.

P280 - Wear protective gloves/protective clothing/eye protection/face protection/ hearing protection.

P308+P313 IF exposed or concerned: Get medical advice/attention.

P501 Dispose of contents/container in accordance with local/ Regional /national /international regulations.

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 Calcium 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 Calcium 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

The reagent is liquid ready for use. After opening, the reagent is stable for 30 days if closed and stored at 2-8°C. Do not mix different batches. A slight variation in the color among batches, 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 R/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):	660 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	Calibr. Serum	Sample
Reagent	µL	1000	1000	1000
Calibr. serum	µL	-	20	-
Sample	µL	-	-	20
Blank	µL	20	-	-

Mix well and incubate for 5 minutes at 37°C.

Reading:

Measure absorbance of the sample and standard against reagent blank.

The reaction volumes may be varied proportionally without alteration of results.

Results:

Manual Method

Calculatin of Calcio:

$$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator serum}} \times \text{Calibrator serum Concentration} = \text{Calcium mg/dL}$$

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 or plasma:

- 8.6 – 10.3 mg/dL (2.15 – 2.57 mmol/L).

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Each laboratory must establish its own normal values on the basis of its local catchment area.

ANALYTICAL CHARACTERISTICS/PERFORMANCE

Linearity

The method is linear up to 26.33 mg/dL (6.57 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.

Accuracy – Recovery

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

Serum– Plasma

Range	Replicates	Mean (mg/dL)	DS	CV%	Recovery
Low	5	10,22	0,000	0,00	99,2%
High	5	13,26	0,182	1,37	115,3%

Urine

Range	Replicates	Mean (mg/dL)	DS	CV%	Recovery
Low	5	7,83	0,276	3,50	97,8%
High	5	11,83	0,740	6,30	98,5%

Interferences

Interference	Limits
Bilirubin	**58 mg/dL**
Haemoglobin	**1000 mg/dL**

The high dilution of the sample with the reagent minimizes interference due to lipids. Copper can interfere with this method, so it is suggested to avoid contamination with reagents with a high presence of copper such as Biuret for reading total proteins. For other interferent substances, refer to the Bibliography.

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	9.40	0.16	1.75	30
High	mg/dL	13.00	0.13	1.33	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	8.92	0.12	1.41	20
High	mg/dL	12.5	0.21	1.69	20

Limit of Sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 0.41 mg/dL (0.10 mmol/L) of Calcium in the conditions established for this test.

Comparison between methods

The method was compared with a similar commercially available method, analyzing 200 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	0.909
Correlation Coeff. (R)	0.937

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. **Butris CA and Ashwood ER** (Ed.). Tietz Fundamentals of Clinical Chemistry. 5th Edition. W.B. Saunders Company. Philadelphia. 2001. p.797-799. 968.
2. **Janssen JW and Helbing AR** (1991). Arsenazo III. An improvement of the routine calcium determination in serum. Eur. J. Clin. Chem. Clin. Biochem. 29 (3) pp. 197-201.1991.
3. **Guder WG. Narayanan S. Wisser H. Zavata** (1996) B. List of analyses; preanalitycal variables. Brochure in: Samples: from patient to the laboratory. Git Verlag GmbH. Darmstadt.
4. **Young D**. (2000) Effects of drugs on clinical laboratory tests. 5th Edition. AACC Press. Washington. DC. 3-149 – 3-158. 2000.
5. **Clinical Laboratory Standards Institute (CLSI).** User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition. EP15-A2. Vol 25 N. 17
6. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2. Vol 24 N. 25
7. **Clinical Laboratory Standards Institute (CLSI).** Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. EP09-A3. Vol 33 N. 11
8. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17
9. **Clinical Laboratory Standards Institute (CLSI).** Interference Testing in Clinical Chemistry, – Third Edition. - EP07.
10. **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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