Amylase – Method CNPG3

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182555	n° 8 vials x 5mL
	REF B75182556	n° 12 vials x 20 mL
CHEMILAB	REF B81180181	n° 3 vials x 26 mL
	REF B81180182	n° 5 vials x 31 mL

INTENDED USE

Product for use in the quantitative determination in vitro of the Amylase activity in human urine, serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

For many years, the levels of serum and plasma α-amylase in patients have provided needed evidence for the diagnosis of the acute pancreatitis. Early assay techniques were based on either a change in the absorption maxima of the complex between starch and iodine as the a-amylase degraded the starch; or a measurement of the increase in reducing groups as the starch was hydrolyzed by the a-amylase. These methods are not as reliable and easy to quantitate as spectrophotometric methods using a defined substrate. Some methods are based on the production of NADH proportionate to the activity of the α-amylase. A defined substrate, such as maltotetraose, is degraded by α-amylase to produce glucose which can be measured in a coupled enzyme assay.

PRINCIPLE OF THE METHOD

Method $CNPG_3$.The direct Amylase assay involves the use of a chromogenic substrate, 2-chloro-4-nitrophenol linked with maltotriose. α-amylase hydrolyzes the 2-chloro-4-nitrophenyl- a-D-maltotrioside (CNPG3) to release 2-chloro-aminophenol (CNP) and form 2-chloro-4-nitrophenyl- a-D-maltoside (CNPG2), maltotriose (G3) and glucose (G). The rate of formation of the 2-chloro-4-nitrophenol can be detected spectrophotometrically at 405 nm to give a direct measurament of a-amylase activity in the sample. The reaction is not readily inhibited by endogeneous factors.

Storage and stability

= storage temperature 2-8°C

If stored closed at the indicated temperature, avoiding direct light, the intact reagents are stable until the expiration date, printed on the label.

Concentrations

Reagente:			
	Conc.	U.M.	
CNPG3	2.27	mmol/L	*GHS08
Sodium Chloride	300	mmol/L	
Calcium Acetate	5.00	mmol/L	
Potassium sulphacyanide	750	mmol/L	
Sodium Azide	< 0.1	%	
MES pH 6.0 ± 0.2	80.0	mmol/L	

*Signal word:**WARNING**

Contains: Potassium thiocyanate (CAS 333-20-0)

H373 - May cause damage to organs through prolonged or repeated exposure.
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P314 - Get medical advice/attention if you feel unwell.
P501 - Dispose of contents/container in accordance with local / regional / national / international regulations.
EUH032 - Contact with acids liberates very toxic gas.

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

Please inform the manufacturer (through your distributor) and the competent authority of the member state of the European Union in which the user and/or patient is established, of cases of serious incident that has occurred in relation to the device. For other jurisdictions, reports of serious incidents must be made in accordance with the regulatory requirements of the home Member State. By reporting serious incidents, you help provide more information about the safety of your in vitro medical diagnostic device.

PROCEDURE

Quality control

Control sera with a known titer of Amylase 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 α-Amylase traceability is reported in the package insert supplied with the "Calibrator Serum".

SAMPLE COLLECTION

Type of sample and storage

Serum or heparanized plasma are recommended sample types. Other anti-coagulants such as EDTA or citrate should not be used. Centrifuge and remove the serum as soon as possible after collection. If not analyzed promptly, samples should be stored at 2-8°C. α-amylase is reported to be stable for up to one week at room temperature (20-25°C) and several months when capped and stored at 2-8°C.

PREPARATION OF THE REAGENT

Reagent liquid ready for use. After opening, the reagent is stable for 30 days when properly capped immediately after each opening and stored at 2-8°C. Slight variations in colour among batches will 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):	405 nm
Temperature:	37°C
Reaction	Kinetic (Increasing Reaction)

Technique -

Bring the reagents to reaction temperature away from direct light.

	U.M.	Blank	Calibr. Serum	Sample
Reagent	µL	1000	1000	1000
Calibr. Serum	µL	-	25	-
Sample	µL	-	-	25

Mix gently and incubate the reaction temperature for 60 seconds. After the incubation, read the absorbance at 405 nm. Repeat at readings at 30 seconds or 1-minute intervals. Recording a minimum of 3 absorbance changes is recommended. Determinate the mean Δ O.D. /min.

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

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Results:

Manual Method

Calculation of Amylase concentration:

$$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator Serum}} \times \text{Calibr. Serum Concentration} = \text{Amylase U/L}$$

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.

Calculation of the results obtained using a multiplication factor

Δ O.D./min x K-factor* = U/L of α-Amylase

Explanation of the calculation:

$$\frac{Vt \times 1000}{M.E.C. \times O.P. \times Vc} \times 2 = K - \text{factor} * \times \Delta O.D./\text{min.} = \text{U/L Amylase}$$

*K-factor = 3178

where:

U/L = activity in serum, in international units

DO.D./min. = variation in absorbance per minute

Vt = total reaction volume (ml)

1000 = conversion to the concentration per liter

M.E.C.= micromolar extinction coef. CNP at pH 6.0 37°C; 12.9 cm^2/mmol at 405 nm

O.P. = optic path (1cm)

Vc = sample volume in the mixture (mL)

REFERENCE RANGE

- Serum or plasma: 35-140 U/L
- Urine: 17-595 U/L (Male) – 19-420 U/L (Female)

Each laboratory should calculate its own normal values on the basis of its local population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE

Linearity

The α-amylase Test Reagent is linear to 1000 U/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.

Recovery

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

Serum – Plasma

Range	Replicates	Mean (U/L)	DS	CV%	Recovery
Low	5	41.0	0.707	1.38	90.3%
High	5	286.8	4.087	1.42	104.3%

Urine

Range	Replicates	Mean (U/L)	DS	CV%	Recovery
Low	5	40.3	7.24	3.7	122%
High	5	196.7	6.27	16.6	93.6%

Interference

The high dilution of the sample with the reagent minimizes interference due to lipids.

Interference	Limit
Bilirubin	**25 mg/dL**
Triglycerides	**600 mg/dL**
Haemoglobin	**500 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	54.5	2.50	4.58	20
High	U/L	1184	4.40	52.1	20
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	50.7	3.09	6.10	20
High	U/L	1125	47.2	4.20	20

Limit of Sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable activity for α–Amylase is 12 U/L at 37°C.

Comparison between methods

The Sclavo method for Amylase was compared with a similar commercially available method, analyzing 111 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-3.6
Slope	0.956
Correlation Coeff. (R)	0.998

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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