Pancreatic Amylase – EPS-G7 Method

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182575	n° 8 vials x 4 mL (R.A) n° 2 vials x 5 mL (R.B)
CHEMILAB	REF B81180191	n° 1 vials x 32 mL (R.A) n° 1 vials x 8 mL (R.B)

INTENDED USE

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

CLINICAL SIGNIFICANCE

α-amylase is a hydrolytic enzyme that splits starches in maltose. In the human body, α-amylase is produced by various organs. Pancreatic amylase is produced by the pancreas and released into the intestinal tract, and saliva amylase is synthesized by the salivary glands and secreted in saliva. Considering that pancreatic amylase and saliva amylase are 97% structurally homologous, the only method for distinguishing them from one another is the utilization of a test based on monoclonal antibodies to inhibit the saliva enzyme. Amylase is eliminated from the blood through the kidneys in urine. Therefore, an increase in serum activity is reflected in an increase in urinary amylase. Measurement of serum and urine α-amylase is used mainly in the diagnosis of pancreatic disorders.

PRINCIPLE OF THE METHOD

Photometric enzymatic test, in which the 4.6-ethylidene-(G7)-p-nitrophenol-(G1) – D-maltoheptaoside (EPS-G7) substrate is split by α-amylase into separate fragments. These fragments are further hydrolyzed in a second phase by α-glucosidase, which produces glucose and p-nitrophenol. Since the saliva isoenzyme is selectively inhibited by a combination of two monoclonal antibodies during the pre-incubation phase, increased absorbency represents pancreatic amylase activity in the sample.

$$5\,EPS\text{-}G_7 + 5\,H_2O \xrightarrow{\alpha Amilasi} 2etileneG_5 + 2G_2PNP + 2etileneG_4 + 2G_3PNP + 2etileneG_5 + 2G_4PNP$$

$$2\text{-}G_2PNP + 2\text{-}G_3PNP + G_4PNP + 14\,H_2O \xrightarrow{\alpha\text{-}Glucosidase} 5PNP + 14G$$

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.

Concentration

Reagent A		
	Conc.	**U.M.**
Good's buffer pH 7,15	0.1	mol/L
NaCl	62.5	mmol/L
$MgCl_2$	12.5	mmol/L
α-Glucosidase	≥ 2.5	kU/L
Monoclonal antibodies	≥ 31	mg/L
Reagent B		
Good's buffer pH 7,15	0.1	mol/L
EPS-G7	8.5	mmol/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

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 Pancreatic 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, utilize the kit "Calibration serum" Sclavo Code B35181702.

Traceability

The Pancreatic amylase traceability is indicated in the calibration serum package insert.

SAMPLE

Sample types and storage

Serum and plasma with heparin or EDTA.

In serum / plasma	7 days	a	20-25 °C
	7 days	a	4-8 °C
	1 year	a	-20 °C
Urine	2 days	a	20-25 °C
	10 days	a	4-8 °C
	3 weeks	a	-20 °C

PREPARATION OF THE REAGENT

Reagents are liquid ready to use. Slight variations in color from batch to batch, will not affect test results. After opening, reagents remain stable until the expiration date if kept in the conditions indicated below in "Storage and stability".

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):	405 nm
Temperature:	37°C
Reaction	End point (increasing reaction)

Technique – Monoreagent procedure

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

	U.M.	Blank	Calib. Serum	Sample
Reagent A	µL	1000	1000	1000
Calib. Serum	µL	-	20	-
Sample	µL	-	-	20
Water	µL	20	-	-
Mix well, incubate at 37°C for 3 min. and add				
Reagent B	µL	250	250	250

Mix well and after 2 minutes carry out reading at 37°C. Read the absorbance of sample (O.D. sample) and calibrator serum (O.D. calibr. serum) against reagent blank (complete reading within 3 minutes).

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

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Results:

Manual Method

Calculation of Pancreatic amylase concentration:

$$\frac{\Delta \text{ D.O. Sample}}{\Delta \text{ D.O. Calibration Serum}} \times \text{Calibrator Serum Conc. (U/L)} = \text{U/L Panc. Amylase.}$$

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 obtained through a special validated algorithm.

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L of Pancreatic amylase
K-factor Reagent B Starter = 5670

REFERENCE RANGE

	Female	Male
Serum/Plasma	< 53 U/L	< 53 U/L
Urine	< 319 U/L	< 356 U/L

Every laboratory must set it own normal-range values based on the population under study.

CHARACTERISTICS/PERFORMANCE

Linearity

Reaction is linear up to 2000 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.

Accuracy - Recovery

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.

Range	Replicates	Mean (U/L)	DS	CV%	Recovery
Low	5	21.2	0.447	2.11	90.2%
High	5	233.0	2.345	1.01	95.9%

Interference

Hemoglobin also interferes at minimum concentrations.

Interference	Limits
Asorbic acid	**30 mg/dL**
Bilirubin	**40 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	mg/dL	69.7	2.18	3.13	20
High	mg/dL	370	3.36	0.91	20
Total precision (Within-lab precision)					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	mg/dL	68.3	1.48	2.17	20
High	mg/dL	371	3.14	0.85	20

Limits of sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 5 U/L of Pancreatic amylase in the conditions established for this test.

Comparison between methods

The Sclavo method for Pancreatic Amylase was compared with a similar commercially available method, analyzing 58 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-1.66
Slope	0.97
Correlation Coeff. (R)	0.994

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

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2. **Wroblewsky F., Ladue J.S.,** (1965). Proc. Soc. Exper. Biol and Med, 91:569
3. **NCCLS Document,** "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. **EU-Dir 1999/11** Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
5. **Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness**; Approved Guideline – Second Edition. EP15-A2.
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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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