Gamma-GT – method Szasz

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182553	n° 10 vials x 16 mL (R.A) n° 2 vials x 21 mL (R.B)
	REF B75182554	n° 15 vials x 4 mL (R.A) n° 1 vials x 16 mL (R.B)
CHEMILAB	REF B81180271	n° 2 vials x 33 mL (R.A) n° 2 vials x 8 mL (R.B)
	REF B81180272	n° 7 vials x 33 mL (R.A) n° 5 vials x 11 mL (R.B)

INTENDED USE

Product for use in the quantitative determination in vitro of the Gamma-glutamyl transferase (γ-GT) activity in human serum. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

Although γ-GT is present in many tissues, the enzyme which we wish to detect in the serum is principally part of the hepato-biliary system. Consequently, raised levels of γ-GT are seen in all forms of disease or damage to the hepatic system. From the clinical viewpoint, the enzyme is useful for the diagnosis of obstructive jaundice, cholangitis, and cholecystitis. High γ-GT levels are also seen during the assumption of alcohol or pharmaceutical products (sedatives, anticonvulsants, and tranquillizers).

PRINCIPLE OF THE METHOD

Method Enzymatic-Kinetic Szasz. In 1963 Orlowski described a method for the determination of y-GT utilizing y-glutamyl-4-nitroanilide as substrate, facilitating the colorimetric determination of enzyme activity by measuring the absorbance of 4-nitroaniline at 405 nm. In 1969 Szasz adapted the use of the substrate to a kinetic photometric method. In 1974 Szasz proposed the use of y-glutamyl-3-carbossy-nitroanilide as substrate. The method described by Szasz in 1969 is recommended by the Scandinavian Society for Clinical Chemistry and Clinical Physiology. The -GT test Sclavo is based upon the recommendations of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Gamma glutamyl transpeptidase (-GT) catalyzes the transfer of the g-glutamyl group from the substrate g-glutamyl-3-carboxy-4-nitroanilide to glycylglycine releasing L-glutamyl-glycylglycine and 5-amino-2-nitrobenzoato.

L-Gamma-glutamyl-3-carboxy-4-nitranilide + Glycylglycine < *Gamma-GT* >
Gamma-glutamyl-glycylglycine + 5-Amino-2-nitrobenzoate

The rate of formation of 5-amino-2-nitrobenzoate, determined kinetically at 405 nm, is proportional to y-GT activity.

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	20	mmol/L	
y-glutamyl-3-carboxi-4-nitroanilide	4.00	mmol/L	
Reagent B:			
Glycylglycine	750	mmol/L	*GHS07

*Signal word: **WARNING**
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P264 Wash thoroughly after handling.
P280 Wear eye protection / face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313 If skin irritation occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before reuse.
P337+P313 If eye irritation persists

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 y-GT 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 γ-GT traceability is reported in the package insert supplied with the "Calibrator Serum".

SAMPLE

Type of sample and storage

Use serum or plasma with EDTA, heparin, citrate or oxalate/fluoride. Use serum free of haemolysis. y-GT is stable in serum for at least a week if stored at -4°C to +20°C and 3 months at -20°C.

PREPARATION OF THE REAGENT

Add 1 volume of Reagent B to 4 volumes of Reagent A and mix gently. The working reagent is stable for at least 30 days at 2-8 ° C and protected from light. Do not use the working reagent if it has a D.O. initial greater than 0.900 against distilled water at 405 nm. A slight variation in the colour 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 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:	405 nm
Temperature:	37°C
Reaction	Kinetic (Increasing reaction)

Technique - Monoreactive

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

-	U.M.	Calibrator Serum	Sample
Reagent	µL	1000	1000
Calib. Serum	µL	100	-
Sample	µL	-	100

Mix gently and incubate at reaction temperature (37°C) for 60 sec.
After the incubation, read the absorbance at 405 nm. Repeat readings at 1-minute intervals. Recording a minimum of 3 absorbance changes is recommended. Determine the mean ΔO.D./min.
Reaction volumes may be varied proportionally without alteration of results.

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Results:
Manual Method
Calculation of γ-GT concentration

$$\frac{\Delta \text{O.D. sample}}{\Delta \text{O.D. Calib.serum}} \text{ x Calib.serum conc.(U/L)} = \text{U/L of y-GT}$$

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 y-GT
Explanation of the calculation:

$$\frac{\text{Vt x 1000}}{\text{M.E.C. x O.P. x Vc}} = \text{K – factor * x } \Delta \text{O.D./min.} = \text{U/L y-GT}$$

*K-factor = 1111
where:
U/L = activity in serum in international units
Δ O.D./min. = variation in absorbance per minute
Vt = total reaction volume (µL)
1000 = conversion to the concentration per liter
M.E.C. = micromolar extinction coefficient of 4-nitroanilide 9.9 cm^2/ µmol at 405 nm
O.P. = optic path (1cm)
Vc = sample volume in the mixture (µl)

REFERENCE RANGE

	Female	Male
Children / Adolescents		
1 day – 6 months	15-132 U/L	12-122 U/L
6 months – 1 year	1-39 U/L	1-39 U/L
1 -12 years	4-22 U/L	3-22 U/L
13 – 18 years	4-24 U/L	2-42 U/L
Adults	< 38 U/L	< 55 U/L

Each laboratory should calculate its own normal values based on its local population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE
Linearity
The method is linear up to the 900 U/L of γ-GT. 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 kit in question following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicates	Mean	DS	CV%	Recovery
Low	5	28.0	0.00	0.00	108.5 %
High	5	121.0	1.09	0.90	100.7 %

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

Interference	Limits
Ascorbic Acid	**30 mg/dL**
Bilirubin	**40 mg/dL**
Haemoglobin	**400 mg/dL**
Triglycerides	**2000 mg/dL**

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	43.6	1.30	3.10	20
High	U/L	153.3	2.90	1.90	20
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	46.3	1.70	3.70	20
High	U/L	87.4	2.50	2.90	20

Limit of Sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. Under the conditions established for this test the lowest detectable concentration is approximately 1.7 U/L of Gamma GT.

Comparison between methods
The proposed method was compared with another commercially available method following the guidelines of the CLSI. analyzing 20 human sera. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	2.0
Slope	0.94
Correlation Coeff. (R)	0.989

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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4. **Nielsen L.G., Ash MO (1978)** Adoption of a manual kineitic gamma-glutamyl transferase method in the clinical laboratory. Amer J Med Technol; 44: 279-85
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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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