AST / GOT – Instructions for use (IFU)

Distributed by: ISE S.r.l. CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

R1: 4 x 50 mL – R2: 4 x 20 mL • REF A-R0200001101

R1: 3 x 18,5 mL – R2: 1 x 18,5 mL • REF R3330000009

IVD CE

INTENDED USE

Product for use in the quantitative determination in vitro of the GOT activity 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

Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) belong to the group of aminotransferases or transaminases; these catalyze the reversible transformation of α-cheto-acids into aminoacids through the transfer of amino groups. AST and ALT are present in human plasma, bile, cerebrospinal fluid and saliva. The serum ALT and AST levels are increased in viral hepatitis and in other forms of hepatic disease. In spite of the fact that, in such cases, both enzymes show raised serum levels, ALT is the more specific enzyme for the diagnosis of hepatic damage. AST levels can rise in connection with cardiac or skeletal muscle damage, in addition to hepatic parenchymal tissue damage.

PRINCIPLE

In the presence of 2-oxoglutarate, aspartate is transformed into oxalacetate and glutamate by the aspartate aminotransferase (AST/GPT) present in the sample. In the presence of NADH and malate-dehydrogenase (MDH), oxalacetate is transformed into malate and NAD. The consumption of NADH over a given period of time, determined at λ 340 nm, is proportional to the GOT concentration in the test sample.

$$L-Aspartate+2-Oxglutarate \xleftrightarrow{AST} L-Glutamate+Oxalacetate$$

$$Oxalacetate+NADH+H^{+} \xleftrightarrow{MDH} D-Malate+NAD^{+}$$

Abbreviations:

AST: Aspartate aminotransferase
MDH: Malate dehydrogenase
NADH: Reduced Nicotinamide-adenine dinucleotide
NAD+: Oxidated Nicotinamide-adenine dinucleotide

REAGENTS

A-R0200001101 – R1: 4 x 50 mL – R2: 4 x 20 mL
Reagent 1: n° 4 vials x 47,0 mL ready for use
Reagent 2: n° 4 vials x 5,5 mL ready for use
R3330000009 – R1: 3 x 18,5 mL – R2: 1 x 18,5 mL
Reagent 1: n° 3 vials x 12,6 mL ready for use
Reagent 2: n° 1 vials x 4,0 mL ready for use

Concentrations

Reagent 1:		
	Conc.	**U.M.**
TRIS buffer pH 8.1± 0.2	88.0	mM
L-Aspartate	265	mM
MDH	≥ 462	U/L
LDH	≥ 660	U/L
2-Oxoglutarate	13.2	mM
Sodium azide	30.0	mM
Reagent 2:		
	Conc.	**U.M.**
TRIS buffer pH 10.2 ± 0.2	10.0	mM
NADH	2.60	mM
Sodium azide	30.0	mM

Precautions

Kit for professional laboratory use, used only by qualified and properly trained technical personnel, under the supervision of a doctor in charge of the laboratory. In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC 1272/2008 Regulation and subsequent amendments and additions. However, it is recommended to handle reagents according to the standards of good laboratory practice.

Reports 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.

Storage and stability

Store at 2 - 8°C and protect from direct light. When correctly stored, the reagent is stable up to the expiry date reported on the label. A slight variation in the composition of the components may occur between batches, but this has no effect on the test results.
After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protected from contamination, evaporation, direct light and stored at correct temperature

SAMPLE COLLECTION

Type of sample and storage

Fresh non-hemolyzed serum or heparinized plasma samples should be used. AST is stable in serum or plasma for 7 days at 4 - 8°C and 12 months at - 20°C (3).

Precautions

All human samples must be handled and disposed of as potentially infectious materials.

Procedure

Quality control

Control sera with known AST level are commercially available for quality control, including certificates of analysis showing the values and limits of confidence. Normal and pathological control sera are available as "Normal control serum" cod. R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:

- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation

Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".
All applications that are not explicitly approved cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user.

Calibration

For calibration use the "Multicalibrator" kit. R0300000006. For ISE srl instruments calibration every 10 days is recommended.

Traceability:

The AST value is visible in the insert of the calibration serum package.

Reaction Conditions

Wavelength (primary): 340 nm
Wavelength (secondary): 380 nm
Temperature: 37°C

Technique - Procedure with Reagent B as starter

Bring the reagents to the reaction temperature

	U.M.	**Calib.Serum**	**Sample**
Reagent A	µL	1000	1000
Calib. Serum	µL	85	-
Sample	µL	-	85
Mix, after 2 minutes add:			
Reagent B	µL	100	100

Mix gently and incubate at 37°C. After incubation, add reagent B and read absorbance at 340 nm after 30 seconds. Repeat readings every 30 seconds or every 60 seconds. At least 3 repetitions of reading in the chosen times are recommended. Mean between Δ D.O./min.

The reaction volumes can be varied proportionately, the calculation remaining unchanged.

Results

The concentration of AST-GOT is obtained by the following formula:

$$\frac{\Delta \text{ D.O. Sample}}{\Delta \text{ D.O. Calib. Serum.}} \times \text{conc. Calib. Serum (U/L)} = \text{U/L di AST-GOT}$$

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L di AST-GOT

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$$\frac{Vt \times 1000}{C.M.E. \times P.O. \times Vc} K - Factor * \times \Delta D.O./min. = U/L \text{ di AST - GOT}$$

*K-factor (Monoreagent method) = 2090
*K-factor (Bireagent method) = 1961
dove:
U/L = activities in international units per liter
Δ D.O./min. = change in absorbance per minute
Vt = total reaction volume (µL)
1000 = conversion of concentration per litre
C.M.E. = coeff. NADH extinction micromolar 6.22 cm2/µmol at 340 nm
P.O. = optical path (1.0 cm)
Vc = sample volume in the final reaction mixture (µL)

NORMAL VALUES
Serum or Plasma:
- Male: < 37.0 U/L
- Female: < 31.0 U/L

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 the following values of 400 U/L at 340 nm.

Specificity
The method is specific for the determination of AST/GOT. Any eventual non-specific reaction terminates within the delay period before taking the reading.

Accuracy – Recovery
The recovery of AST/GOT added to a normal sample at known concentrations showed a result of 94.5%.

Interferences
Triglycerides below 2000 mg/dL does not interfere in the reaction. Ascorbic acid influences the reaction at concentrations over 30 mg/dL.

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	42.6	1.82	4.28	20
High	U/L	12.3	3.84	1.38	20
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	42.6	1.19	2.81	20
High	U/L	12.3	11.7	4.20	20

Sensitivity
At λ 340 nm a concentration of about 5.93 U/L of AST/GOT in the conditions established for this test.

Comparative method
The AST/GOT method was compared with a similar method as described by the IFCC optimization (1). Samples tested = no. 60; y intercept = 1.043x + 3.97; Correlation Coefficient r = 0.997.

Disposal of reagents
Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

Manufacturer:
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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. Recommendation on I.F.C.C. methods for measurement of catalytic concentrations of enzymes, Clin Chem, 23:5 (1977).
2. Wroblewsky F., Ladue J.S., Proc. Soc. Exper. Biol and Med, 91:569 (1965).
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.

REVISION	DATE	CHANGE
Rev.A	12/2022	New Issue for IVDR Regulation (UE) 2017/746 compliance