SGOT (IFCC without PDP)

INTENDED USE

Bioline SGOT (AST) is used for the quantitative determination of aspartate aminotransferase (AST) in human serum.

CLINICAL SIGNIFICANCE

Serum Aspartate amino transferase (AST) also known as serum glutamic oxaloacetic transaminase (SGOT) is a tissue enzyme that catalyzes the exchange of amino and keto groups between alpha-amino acids and alpha-ketoacids. AST is widely distributed in tissue principally cardiac, hepatic, muscle and kidney. Injury to these tissues results in the release of the AST (SGOT) enzyme to general circulation. Following a myocardial infarction, serum levels of AST (SGOT) are elevated and reach a peak 48 to 60 hours after onset. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also will increase serum AST levels.

METHOD AND PRINCIPLE

The first kinetic assay of AST for diagnostic purposes was described by Karmenetal .in 1955, using a coupled reaction of Malate dehydrogenase (MDH) and NADH. This assay system was critically evaluated and optimized in 1960 by Henryetal. In 1977 the International Federation of Clinical Chemistry recommended a reference procedure for the measurement of AST activity based upon Karmen's procedures. The AST reagent applies the formulation recommended by the IFCC.

The enzymatic reaction sequence employed in the assay of Aspartate amino transferase is as follows:

$$\text{L-Aspartate+2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate+L-Glutamate}$$

$$\text{Oxalacetate+NADH+H}^{+} \xrightarrow{\text{MDH}} \text{Malate+NAD}^{+}\text{+H}_2\text{O}$$

AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate.The Oxalacetate formed in the first reaction is then reacted with NADH in the presence of Malate dehydrogenase (MDH) to form NAD. AST activity is determined by measuring the rate of oxidation of NADH at 340 nm. Lactate dehydrogenase is included in the reagent to convert endogenous pyruvate in the sample to lactate during the lag phase prior to measurement.

REAGENT COMPOSITION

AST Liquid Reagents 1 and 2 come in separate containers, and both reagents are clear, colorless liquid in ready to use format. After combining AST Liquid R1 (Buffer Reagent) and AST Liquid R2 (Co-Enzyme) the reagent contains:

L-Aspartate	240mmol/L
MDH	>600 U/L
LDH	>600 U/L
TrisBuffer,pH7.5	80 mmol/L
2–Oxoglutarate	12mmol/L
NADH	0.18mmol/L
Stabilizers and Preservatives	

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.

CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.

Specimens should be considered infectious and handled appropriately.

2. Use distilled or deionized water where indicated.

WORKING REAGENT PREPARATION

The working reagent is prepared by mixing 4 volumes of R1 with 1 volume of R2 in a disposable container.

Example: 0.8 ml R1 + 0.2ml R2

REAGENT STORAGE AND STABILITY

Reagents are stable until the expiration date on their respective labels, when properly stored at 2 - 8°C and protected from light. Reagents should appear clear and colorless. The working reagent is stable for 4 weeks at 2 - 8°C.

REAGENT DETERIORATION

1. Discard the reagent if it appears turbid or contains particulate matter.
2. The reagent fails to meet linearity claims or fails to recover Control values in the stated range.
3. The reconstituted reagent has a reagent blank absorbance less than 0.900 at 340 nm.

SPECIMEN COLLECTION AND STORAGE

Non-hemolyzed serum is the specimen of choice, yet EDTA treated plasma or lithium heparinized plasma can be used. Whenever possible, specimens should be separated and analyzed on the day of collection.Store serum in stoppered tubes.The enzyme in serum is reportedly stable for 7 days at 2-8°C and 3 months at -20 deg c.

INTERFERENCES

Hemolysis must be avoided as the concentration of AST in red cells is roughly 10 times that of serum. Bilirubin levels up to 40 mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test .Certain drugs and other substances are also known to affect AST values.

ASSAY PROCEDURE FOR SEMIAUTO ANALZYER

Wavelength :340 nm

Temperature : 37°C

	Test
ReagentR1	800µL
ReagentR2	200µL
Sample	50µL

Mix and aspirate into the analyzer, after 1 minute delay measure the change of optical density per minute (ΔOD/min.) during next 2 minutes

High Linearity Procedure

For Samples above 800 IU/L record the change in OD per min for 60 Sec only, after a delay of 30 sec and multiply with the factor. With this procedure linearity of 1600 IU/L can be achieved.

CALCULATION

SGOT (AST) IU/L= ΔA/Min X3336

LIMITATIONS

Using normal manual procedure ,reagent is linear upto 800 IU/L. For Sample that have AST values greater than 800 IU/L follow the High Linearity Procedure.

QUALITY CONTROL

It is recommended that control be included in each set of assays .Commercially available control material with established AST values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control

material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

Normal Range: 8 – 45 U/L (37°C)

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments ,laboratories ,and local populations.

PERFORMANCE CHARACTERISTICS

Comparison: A group of 125 sera ranging in AST activity from 13 - 399 U/L was assayed by the described AST method and by a similar commercially available AST reagent. Comparison of the results yielded a correlation coefficient of 0.999 and the regression equation was y =0.964x+0.964.

Linearity: Linear upto 800 U/L at 37°C. For samples above the linearity range dilute with normal saline and rerun. Multiply the obtained result with the dilution factor to get correct result.

Precision: Within run

Mean (IU/L)	S.D.	C.V.%
41.7	0.9	2.2
115	3.3	2.9

Precision: Between run

Mean (IU/L)	S.D.	C.V.%
42.4	1.5	3.1
118	3.8	3.3

GENERAL TECHNICAL PARAMETERS

Mode	Kinetic
Wavelength (Filter)	340nm
Reaction Direction	Decreasing
Sample Vol.	50 µl
Reagent Vol.	1000 µl
Delay Time / Lag Time	60 Sec
Interval Time	60 Sec
No. of reading	2
Measuring time	120 Sec
Reagent Blank Abs.(Max)	NLT 0.9
Calibration Method	Fix factor
Factor	3336
Linearity	800 IU/L
High Linearity	1600 IU/L
Decimal Places	1
Temp.	37°C
Unit	IU/L
Ref. Low (Male / Female)	8 IU/L
Ref. High (Male / Female)	45 IU/L

REFERENCES

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