

Intended Use

For the quantitative determination of Aspartate Aminotransferase (AST) in human serum.

Clinical Significance

AST is widely distributed in tissues with the highest concentrations found in the liver, heart, skeletal muscle and kidneys. Diseases involving any of these tissues can lead to elevated levels of AST in serum. Following myocardial infarction, AST levels are elevated and reach a peak after 48 to 60 hours.

Hepatobiliary diseases such as cirrhosis, metastatic carcinoma and viral hepatitis can show increased levels of AST. Other disorders which can lead to an elevated level of AST are muscular dystrophy, dermatomyositis, acute pancreatitis and infectious mononucleosis.¹

Method History

Karmen² developed a kinetic assay procedure in 1955 which was based upon the use of malate dehydrogenase and NADH. Optimized procedures were presented by Henry³ in 1960 and Amador and Wacker⁴ in 1962. These modifications increased accuracy and lowered the effect of interfering substances. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology⁵ published a recommended method based on optimized modifications in 1974. In 1976, the Expert Panel on Enzymes of the International Federation of Clinical Chemistry (IFCC)⁶ proposed the addition of pyridoxal-5-phosphate to the reaction mixture to ensure maximum activity. The IFCC⁷ published a recommended method that included P-5-P in 1978. The present method is based on IFCC recommendations but does not contain P-5-P since most specimens contain adequate amounts of this cofactor for full recovery of AST activity.^{8,9,10}

Principle



Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to L-Ketoglutarate to yield oxalacetate and L-glutamate. The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

Reagents

After combining R1 and R2 the reagent contains: L-aspartic acid 240mM, L-ketoglutaric acid 12mM, LDH (microbial) > 1000U/L, MDH (microbial) >800U/L, NADH 0.18mM, Buffer 80mM, pH 7.8±0.1, Sodium Azide 0.01%, Stabilizers.

Reagent Preparation

The reagents are ready to use for systems capable of handling two reagents. If a single reagent is required, prepare working reagent by mixing 5 parts of R1 reagent with 1 part R2 reagent. (e.g. 250 ul R1 with 50 ul R2 reagent.)

Reagent Storage

1. Store reagents at 2-8°C.
2. Working reagent is stable for 48 hours at room temp. (15-30°C) and for 14 days when refrigerated (2-8°C).

Reagent Deterioration

Do not use reagent if:

1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

Precautions

1. This reagent set is for *in vitro* diagnostic use only.
2. The R1 reagent contains sodium azide (0.01%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Specimen Collection and Storage¹¹

1. Non-hemolyzed serum is recommended. Red cells contain AST which can give falsely elevated results.
2. AST in serum is reported stable for ten days when refrigerated (2-8°C), two weeks when frozen (-20°C), and four days when stored at room temperature (15-30°C).

Interferences

1. A number of drugs and substances affect AST activity. See Young, et al.¹²
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.¹³
3. Bilirubin to at least 18 mg/dl, and hemoglobin to at least 300 mg/dl, have been found to have a negligible effect on this procedure.

Materials Provided

AST (SGOT) Reagents R1 and R2.

Materials Required but not Provided

1. Accurate pipetting devices.
2. Test tubes/rack.
3. Timer.
4. Spectrophotometer able to read at 340 nm. (UV)
5. Heating bath/block (37°C).

Test Procedure (Automated)

Wavelength:	340 nm
Assay Type:	Kinetic
Sample/Reagent Ratio:	1:11
Reaction Direction:	Decreasing
Temperature:	37°C
Lag Time:	60 seconds
Read Time:	60 seconds
Low Normal:	5 U/L
High Normal:	34 U/L

Application Parameters for various automated instruments are available. Please contact the manufacturer's Technical Service Department for specific information.

Test Procedure (Manual)

1. Prepare reagent according to instructions.
2. Pipette 1.0ml of reagent into appropriate tubes and pre-warm at 37°C for five minutes.

Liquid AST (SGOT) Reagent Set

3. Add 0.100ml (100ul) of sample reagent, mix and incubate at 37°C for one minute.
4. After one minute, read and record absorbance at 340nm against a water blank. Return tube to 37°C. Repeat readings every minute for the next two minutes.
5. Calculate the average absorbance difference/minute (Δ Abs./Min.).
6. The Δ Abs./Min. multiplied by the factor 1768 (See Calculation) will yield results in IU/L.

Procedure Notes

1. If the spectrophotometer being used is equipped with a temperature-controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.
2. Turbid or highly icteric samples may give readings whose initial absorbance exceeds the capabilities of the spectrophotometer. More accurate results may be obtained by using 0.05ml (50 ul) of sample and multiplying the final answer by two.

Limitations

1. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.¹³

Calibration

The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Calculation

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$\text{AST (IU/L)} = \frac{\Delta\text{Abs./Min.} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \Delta\text{Abs./min.} \times 1768$$

Where Δ Abs./Min. = Average absorbance change per minute
1000 = Conversion of IU/ml to IU/L
1.10 = Total reaction volume (ml)
6.22 = Millimolar absorptivity of NADH
0.10 = Sample Volume (ml)
1.0 = Light path in cm

Example: If the average absorbance change per minute = 0.12 then $0.12 \times 1768 = 212$ IU/L

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal AST (SGOT) values. These controls should be run at least with every shift in which AST (SGOT) assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values¹³

8 to 22 IU/L (30°C)

5 to 34 IU/L (37°C)

Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance

1. Linearity: 0-500 IU/L.
2. Comparison: Studies between the present method and a similar method yielded a correlation coefficient of 0.999 and a regression equation of $y=0.98x + 1.6$. (n=125, range=15-659 IU/L)
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
42	1.2	2.9	43	1.3	3.0
202	1.7	0.8	206	4.0	1.9
408	2.6	0.6	411	4.4	1.1

4. Sensitivity: The sensitivity for this reagent was investigated by reading the change in absorbance at 340nm for a saline sample and samples with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, the AST (SGOT) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L AST activity gives a Δ Abs./Min. of 0.0004.

References

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