

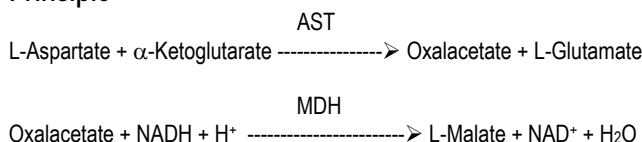
Intended Use

For the quantitative determination of aspartate aminotransferase in serum.

Method History

Karmen¹ developed a kinetic assay procedure in 1955 which was based upon the use of malate dehydrogenase and NADH. Optimized procedures were presented by Henry² in 1960 and Amador and Wacker³ 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 Physiology⁴ 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)⁵ proposed the addition of pyridoxal-5-phosphate to the reaction mixture to ensure maximum activity. The IFCC⁶ 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 the diagnostic significance of increased AST is still under study.^{7,8,9}

Principle



Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α -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

(Concentrations refer to reconstituted reagent):

α -Ketoglutarate 12mM, L-Aspartic Acid >150mM, NADH >0.2mM, LDH >800 U/L, MDH >600 U/L, Tris Buffer, pH 7.8 \pm 0.1. Non-reactive fillers and stabilizers.

Reagent Preparation

Reconstitute vial with the volume of distilled water stated on the vial label. Swirl to dissolve.

Reagent Storage

1. Store dry reagent at 2-8°C.
2. Reconstituted reagent is stable for 48 hours at room temperature and for thirty days when refrigerated immediately.

Reagent Deterioration

Do not use reagent when:

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

Precautions

This reagent is for *in vitro* diagnostic use only.

Specimen Collection and Storage¹⁰

1. Non-hemolyzed serum is recommended. Red cells contain AST.
2. AST in serum is reported stable for ten days when refrigerated, two weeks frozen, and four days when stored at room temperature.

Interferences

A number of drugs and substances affect AST activity. See Young, et al.¹¹

Materials Provided

AST (SGOT) reagent.

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)

Procedure (Automated)

Refer to specific instrument application instructions.

Procedure (Manual)

1. Reconstitute reagent according to instructions.
2. Pipette 1.0 ml of reagent into appropriate tubes and prewarm at 37°C for five minutes.
3. Zero spectrophotometer with water at 340nm.
4. Add 0.100ml (100ul) of sample to reagent, mix and incubate at 37°C for one minute.
5. After one minute read and record the absorbance. Return tube to 37°C. Repeat readings every minute for the next two minutes.
6. Calculate the average absorbance difference per minute (Δ abs./min.)
7. The Δ abs./min. multiplied by the factor 1768 (see Calculations) will yield results in IU/L.
8. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

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 (50ul) of sample and multiplying the final answer by two.

Calibration

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

AST (SGOT) Reagent Set

Calculations

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 $\Delta\text{Abs./Min.}$ = Average absorbance change per minute

1.10 = Total reaction volume (ml)

1000 = Conversion of IU/ml to IU/L

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

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

NOTE: If any of the test parameters are altered, a new factor must be calculated using the above formula.

Quality Control

The validity of the reaction should be monitored by use of control sera with known normal and abnormal AST (SGOT) values.

Expected Values¹²

Up to 28 IU/L (30°C)

Up to 40 IU/L (37°C)

It is strongly recommended that each laboratory establish its own normal range.

Performance

- Linearity: 500 IU/L
- Comparison: Studies between the present method and a similar method yielded a correlation coefficient of 0.999 and a regression equation of $y=1.00x+1.6$.
- Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
31	2.2	7.1	31	2.5	8.1
156	3.2	2.1	160	4.0	2.5

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