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## AST (SGOT) LIQUID REAGENT (KINETIC METHOD)

Catalog Number: BQ006D-CR

### INTENDED USE

For the quantitative determination of aspartate aminotransferase (AST) in human serum. The reagents are used in routine examination and monitoring of therapy and relapses.

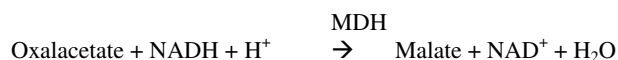
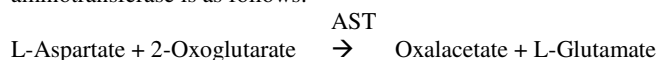
### INTRODUCTION

Serum aspartate aminotransferase (AST) also known as serum glutamic oxalacetic transaminase (SGOT) is a tissue enzyme that catalyzes the exchange of amino and keto groups between alpha-amino acids and alpha-keto acids. 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.<sup>1</sup>

The first kinetic assay of AST for diagnostic purposes was described by Karmen et al. in 1955, using a coupled reaction of malate dehydrogenase (MDH) and NADH.<sup>2</sup> This assay system was critically evaluated and optimized in 1960 by Henry et al.<sup>3</sup> In 1977 the International Federation of Clinical Chemistry recommended a reference procedure for the measurement of AST activity based upon Karmen's procedures.<sup>4</sup> The AST reagent applies the formulation recommended by the IFCC.

### PRINCIPLE

The enzymatic reaction sequence employed in the assay of aspartate aminotransferase is as follows:



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 NADH 0.18 mmol/L Stabilizers and Preservatives reagent contains:

L-Aspartate	240 mmol/L
MDH (porcine muscle)	> 600 U/L
LDH (rabbit muscle)	> 600 U/L
Tris Buffer, pH 7.5	80 mmol/L
2 – Oxoglutarate	12 mmol/L
NADH	0.18 mmol/L
Stabilizers and Preservatives	

### WARNINGS AND PRECAUTIONS

The reagents are for in vitro diagnostic use. Normal precautions exercised in handling laboratory reagents should be followed. The reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

### REAGENT PREPARATION

The working reagent is prepared by mixing five (5) volumes of R1 with one (1) volume of R2 in a disposable container.

Example: 25 ml R1 + 5 ml 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. Discard if either appears cloudy or contains particulate matter. The working reagent is stable for 4 weeks at 2 – 8 °C. The working reagent should be discarded if the initial absorbance, read against distilled water at 340 nm, is below 1.100.

### MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer capable of absorbance reading at 340 nm and 1cm light path
2. Constant temperature block or bath, 37 °C, or temperature controlled cuvette well
3. Accurate pipetting devices
4. Test tubes
5. Interval timer

### SPECIMEN COLLECTION AND STORAGE

Non-hemolyzed serum is the specimen of choice, yet EDTA treated plasma or heparinized plasma can be used.<sup>5</sup> 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 a minimum of 7 days at 2 – 8 °C.<sup>6</sup>

### INTERFERING SUBSTANCES

Hemolysis must be avoided as the concentration of AST in red cells is roughly 10 times that of serum.<sup>5</sup> 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.<sup>7</sup>

### MANUAL PROCEDURE

1. Prepare AST working reagent according to instructions.
2. Zero spectrophotometer at 340 nm with distilled water.
3. For each sample and control, add 1.0 mL working reagent to cuvette or test tube and warm to 37 °C for 3 minutes.
4. Add 100 µL (0.10 mL) serum to its respective tube and mix gently.
5. Read and record absorbance at 1 minute. Continue incubating at 37 °C and record absorbance again at 2 and 3 minutes. Rate should be constant.
6. Determine the average absorbance per minute (DA/min), multiply by factor 1768 for results in U/L.

NOTE: If cuvette is not temperature controlled, incubate samples at 37°C between readings.

### AUTOMATED PROCEDURE

Refer to appropriate application manual available.

### 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 either reagent deterioration, instrument malfunction, or procedural errors.

## CALIBRATION

AST activity is based on the "micromolar extinction coefficient" of NADH at 340 nm (see "Results" section). The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer. Assaying the AST contents of a control serum with known AST values can be used to assure instrument calibration has been performed correctly.

## RESULTS

Values are derived based on the "absorptivity micromolar extinction coefficient" of NADH at 340 nm (0.00622). Units per liter (U/L) of AST/GOT activity is that amount of enzyme which oxidizes one  $\mu\text{mol/L}$  of NADH per minute.

$$\text{U/L} = \frac{\Delta\text{A/Min}}{\text{Absorptivity}} \times \frac{\text{Total Volume}}{\text{Sample Volume}}$$

$$\text{U/L} = \frac{\Delta\text{A/Min}}{0.00622} \times \frac{1.10}{0.10}$$

$$\text{U/L} = \Delta\text{A/Min} \times 1768$$

## LIMITATIONS

If the DA/min. is greater than 0.342, dilute 1 part sample with 9 parts isotonic saline and re-assay. Multiply the result by 10. AST values for neonatal patients have not been established with this procedure. Grossly icteric or turbid specimen may require the use of a sample blank.

## EXPECTED VALUES<sup>8</sup>

Normal Range: 8 – 33 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  $y = 0.964x + 0.964$ . (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

**Precision:** Within-run precision was established by 30 assays on two different levels of commercial serum controls. Total Precision values were obtained by assaying the two commercial controls for 5 consecutive days.

	<i>Within-Run</i>	
	<u>Serum 1</u>	<u>Serum 2</u>
Mean ALT (U/L)	41.7	115.2
Std. Deviation (U/L)	0.9	3.3
C.V. (%)	2.2	2.9

	<i>Run-to-Run</i>	
	<u>Serum 1</u>	<u>Serum 2</u>
Mean ALT (U/L)	40.4	116.2
Std. Deviation (U/L)	0.87	4.88
C.V. (%)	2.14	4.19

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

**Linearity:** Linear to 500 U/L at 37°C.<sup>6</sup> Performed according to NCCLS Guideline EP6-P.

**Sensitivity:** Based on an instrument resolution of  $A = 0.001$ , the method presented shows a sensitivity of 2.65 U/L.

## REFERENCES

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