AST/GOT Liqui-UV® Test
Procedure No. FT687

For the Kinetic Quantitative Determination of AST/GOT in Serum for Manual and/or Automated Procedures

Summary and Principle

Aspartate aminotransferase (AST) is one of several enzymes that catalyze the exchange of amino and oxo groups between alpha-amino acids and alpha-oxo acids. It is widely distributed throughout body tissues with significant amounts in the heart and liver.[1] Lesser amounts are found in skeletal muscle, kidneys, pancreas, spleen, lungs and brain. Injury to these tissues results in release of the AST enzyme to the general circulation. In myocardial infarction, serum AST may begin to rise within 6-8 hours after onset, peak within two days and return to normal by the fourth or fifth day of post infarction.[2] An increase in serum AST is also found with hepatitis, liver necrosis, cirrhosis and liver metastasis.[3]

Karmen[4] first reported a kinetic method for measuring AST activity in serum. Subsequently, the method has been modified and optimized by Bergmeyer et al.[5] Our assay procedure for AST measurement is similar to the method recommended by the International Federation of Clinical Chemistry.[6] The enzymatic reactions involved in the assay procedure are as follows:

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

$$\text{Oxalacetate} + \text{NADH} + {}^{H+} \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^{+}$$

AST catalyzes the transfer of the amino group aspartate to 2-oxoglutarate to yield oxalacetate and glutamate. 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.

Reagents

AST Buffer (R1), Cat. No. FT687a

Composition:			
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	

AST Enzyme (R2), Cat. No. FT687b

Composition:		
2_- Oxoglutarate	12	mmol/L
NADH (Disodium salt)	0.18	mmol/L

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: Buffer and Enzyme liquid reagents are supplied ready-to-use. Prepare Working Reagent in the ratio of 5 parts Buffer (R1) to 1 part Enzyme (R2) (i.e., 25 mL Buffer and 5 mL Enzyme).

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 or 5 days at room temperature (15-30°C). The Working Reagent should be discarded if the initial absorbance, read against distilled water at 340 nm, is below 0.800.

Material Required But Not Provided

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

Specimen Collection and Storage

Non-hemolyzed serum is the specimen of choice, yet EDTA treated plasma or heparinized plasma can be used.[7] 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.[8]

Interfering Substances: Hemolysis must be avoided as the concentration of AST in red cells is roughly 10 times that of serum.[7] Bilirubin levels up to 40 mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test.[1T] Certain drugs and other substances are also known to affect AST values.[9]

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 uL (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 -1746 for results in U/L.

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

Quality Control: Ser-T-Fy I, Normal Control Serum, Cat. No. FT7670 and Ser-T-Fy II, Abnormal Control Serum, Cat. No. FT7680 are recommended for each run. Other commercially available controls with AST values are assayed by this method are also suitable. AST activity is determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed with each run.

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 (0.0063). Units per liter (U/L) of AST/GOT activity is that amount of enzyme which oxidizes one µmol/L of NADH per minute.

$$U/L = (\Delta A/\text{Min Absorptivity}) \times (\text{Total Volume/Sample Volume})$$
$$U/L = (\Delta A/\text{Min}/0.0063) \times (1.10/0.10)$$

$$U/L = \Delta A/\text{Min} \times 1746$$

Limitations

If the ΔA/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[10]

Normal Range: 8 - 33 U/L (37°C)

This range should serve only as a guideline. 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 62 sera ranging in AST activity from 12 - 463 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 0.993 and the regression equation was y = 0.988x + 0.43. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

Precision: Within-run precision was established by 20 assays on three different levels of commercial serum controls. Total Precision values were obtained by assaying the 3 commercial controls for 5 consecutive days.

	Within-Run		
	Serum 1	**Serum 2**	**Serum 3**
Mean ALT (U/L)	25	51	116
Std. Deviation (U/L)	0.8	1.6	0.9
C.V. (%)	3.3	3.1	0.8
	Total Precision		
	Serum 1	**Serum 2**	**Serum 3**
Mean AST (U/L)	26	49	115
Std. Deviation (U/L)	1.1	0.7	0.8
C.V. (%)	4.4	1.4	0.7

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

Linearity: Linear to 600 U/L at 37°C.[8] Performed according to NCCLS Guideline EP6-P.

Sensitivity: Based on an instrument resolution of A = 0.001, the method presented shows a sensitivity of 1.75 U/L.

References

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11. Interchim Laboratory Data

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