AST

(LIQUID)

3 x 60, 3 x 15 ml

RE – ORDER AST1040

INTENDED USE

This reagent is intended for the quantitative determination of aspartate aminotransferase (AST) in serum.

METHOD AND HISTORY

Karmen developed a kinetic assay procedure in 1955 which was based upon the use of malate dehydrogenase and NADH. Optimized procedures were presented by Henry in 1960 and Amador and Wacker 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 Physiology 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) proposed the addition of pyridoxal-5-phosphate to the reaction mixture to ensure maximum activity. The IFCC 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.

TEST PRINCIPLE

The AST catalyzes the reaction of 2-oxoglutarate and L-aspartate to L-glutamate and oxalacetate. Then malate dehydrogenase (MDH) catalyzes the oxidation of NADH to NAD.

$$\text{L-Aspartate} + \textit{a}\text{-Ketogllutaratae} \xrightarrow{\text{AST}} \text{L-Glutamate} + \text{Oxalacetate}$$

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

Aspartate aminotransferase (AST) catalyzes the tranfer of the amino group from L-aspartate to *a*-Ketogllutarate to yield oxalacetate and Ll-glutamate. The oxalacetate undergoes reduction with simultaneous oxidatiion 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.

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 elevataed 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.

PATIENT PREPARATION

No special patient preparation is required.

SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-200 µl. .

Record the patient's name, date and time of sample collection and preparation.

SPECIMEN STORAGE

Serum samples should be kept refrigerated (2° to 8°C) and analyzed within 24 hours. If this is not possible, serum samples may be stored refrigerated (2° to 8°C) or frozen (-20° to 0° C) and are stable for up to 7 days. Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

MATERIALS

Reagents necessary for the determination of ASTare included in the kit.

REAGENT

AST working reagent contains:

L-aspartic acid	280 mM
LDH	1400 U/L
malate dehydrogenase (pig heart)	1000 U/L
2-oxoglutarate	12 mM
alpha-ketoglutaric acid	14 mM
NADH	1.27 mM
sodium azide	0.01%
buffer, preservative	

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

This reagent contains sodium azide (0.01%) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large amounts of water.

REAGENT PREPARATION

The working reagent is prepared by combing 4 parts of R1 (substrate/enzyme) to 1 part of R2 (coenzyme).

REAGENT STORAGE AND STABILITY

Unopened reagents are stable at 2-8° C (refrigerated) until the expiration date stated on the labels. The working reagent is stable at 2-8° C (refrigerated) for 14 days.

The initial absorbance of the working reagent read against distilled water at 340nm (1 cm pathlength) should be at least 0.8 to be considered suitable for use.

ADDITIONAL MATERIALS REQUIRED

4Spectrophotometer capable of reading absorbance at 340 nm.

41 cm cuvettes or a flow cell capable of transmitting light at 340 nm.

Test tubes and pipettes.

Timer with one minute increments

Constant temperature source which can be adjusted to 37°C.

Normal and abnormal control for quality control.

TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

Application procedures for use on automated analyzers are available. Contact RBI's Technical Service Department for specific information.

PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	37° C
Pathlength	1.0 cm
Mode	kinetic
Lag time	1 min
Sample to reagent ratio	1:10

INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

CALIBRATION

No reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

PROCEDURE

5Prepare the required volume of AST working reagent. (See 4.3 Reagent Preparation section.)

5Into separate test tubes pipette 100 µl of serum to be assayed.

5Add 1.0 ml of reagent, mix, and incubate for one minute at 37° C.

Record the decrease in absorbance at 340 nm at one minute intervals until the absorbance change is constant.

CALCULALTION AND RESULTS

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AST (U/L) =

$$\frac{\Delta A/min \times assay\ volume\ (ml) \times 1000}{6.22 \times light\ path\ (cm) \times sample\ volume\ (ml)} = \Delta A/min \times 1768$$

ΔA/min = change in absorbance per minute
Assay volume = total reaction volume expressed in ml
1000 = converts U/ml to U/L
6.22 = absorbance coefficient of NADH at 340 nm
Light path = length of the light path expressed in cm (usually 1)
Sample volume = sample volume expressed in ml
1768 = factor derived from constants in the equation
Example: AST (U/L) =

$$\frac{.015 \times 1.1 \times 1000}{6.22 \times 1 \times 0.1} = .015 \times 1768 = 27\ U/L$$

0.015 = change in absorbance per minute
1.1 = assay volume in ml
1 = light path in cm
0.1 = sample volume in ml

EXPECTED VALUES

The range of expected values is:
8-22 U/L (30° C)
5 -34 U/L (37° C)
These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

MEDICAL ALERT VALUES

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

LIMITATIONS OF PROCEDURE

This procedure measures total AST. Red blood cells contain high concentrations of AST, therefore hemolysis can elevate results. A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S., et. al.

QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples,new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

CALIBRATION PROCEDURES

No reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

PERFORMANCE CHARACTERISTICS

PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

Mean (U/L)	SD (U/L)	CV (%)
42	1.2	2.9
202	1.7	0.8
408	2.6	0.6

Between-Run

Mean (U/L)	SD (U/L)	CV (%)
43	1.3	3.0
206	4.0	1.9
411	4.4	1.1

CORRELATION

A correlation study was done comparing this method and a similar AST method. The samples range between 15 and 659 U/L.

Number of Samples	Regression Equation y=NBS, x=Comparative	Correlation Coefficient
125	y = 0.98 x +1.6	.999

LINEARITY

This procedure is linear to 500 U/L.
A sample with AST activity exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the result.

SENSITIVITY

An absorbance change of 0.0004 ΔA/min corresponds to approximately 1 U/L AST activity.

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