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AST (SGOT) REAGENT (COLORIMETRIC, ENDPOINT PROCEDURE)

Catalog Number: BQ006A-CR

INTENDED USE

For the quantitative colorimetric determination of AST (SGOT) in serum.

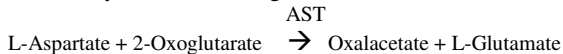
INTRODUCTION

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.¹

Methods for the determination of serum AST (SGOT) include ultraviolet kinetic analysis and colorimetric methods. Earlier colorimetric methods were based on the reaction of oxalacetate with dinitrophenylhydrazine.² However, this reaction is time consuming and non-specific reactions. The present method is based on a modification of the colorimetric method by Doumas and Briggs, which offers increased specificity and shortened incubation time.³

PRINCIPLE

AST catalyzes the following reaction.



In the present method, a diazonium salt is used which selectively reacts with the oxalacetate to produce a color complex that is measured photometrically.

REAGENT COMPOSITION

1. AST (SGOT) Substrate: 33 mM Aspartic acid, 5 mM ketoglutaric acid, phosphate buffer pH 7.4.
2. AST (SGOT) Color Reagent: 0.25% w/v Diazonium salt preserved with formalin.
3. AST (SGOT) Calibrator: A lyophilized serum with AST (SGOT) value provided in each lot.

WARNINGS AND PRECAUTIONS

1. Specimens should be considered infectious and handled appropriately.
2. Exercise the normal precautions required for the handling of all laboratory reagents.
3. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT PREPARATION

Substrate reagent is ready to use. Reconstitute color reagent and calibrator with volume of distilled water indicated on the vial label. Let stand until dissolved and swirl to mix. Calibrator is stable for five days at 2 – 8 °C after reconstitution.

REAGENT STORAGE AND STABILITY

Store AST (SGOT) Substrate, AST (SGOT) Color Reagent, and AST (SGOT) Calibrator in the refrigerator at 2 – 8 °C.

REAGENT DETERIORATION

1. The AST (SGOT) substrate should be a clear colorless solution. Reagent should be discarded if turbidity or discoloration is noted.
2. If AST (SGOT) Color Reagent darkens or if dark brown precipitate is visible, do not use.
3. Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

SPECIMEN COLLECTION

This assay is intended for use with human serum. Reports indicate that AST (SGOT) in serum remains stable at 4°C for a minimum of seven (7) days. Hemolyzed specimens should not be used as erythrocytes contain fifteen times the AST (SGOT) activity in serum.⁴

INTERFERING SUBSTANCES

Pyridoxal phosphate can elevate AST (SGOT) values by activating the apoenzyme form of the transaminase. Pyridoxal phosphate may be found in diluent water contaminated with microbial growth.⁵ High levels of serum pyruvate may also interfere with assay performance. Young et al. give a list of drugs and other substances that interfere with the determination of AST (SGOT) activity.⁶ Refer also to N.E. Saris for a list of references.⁷

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipetting devices
2. Test tubes/rack
3. Timing device
4. Spectrophotometer
5. Heating block or bath (37°C)
6. 0.1 N Hydrochloric Acid

MANUAL ENDPOINT PROCEDURE

1. Place 0.5 ml of AST (SGOT) substrate into test tubes labeled "Blank", "Calibrator", "Control", and "Unknowns". Warm vials in 37 °C heating bath for at least four (4) minutes.
2. At timed intervals, add 0.1 ml (100 µl) of samples into their respective tubes, gently mix, and return to 37 °C heating bath for exactly ten (10) minutes. (Use distilled water for sample blank)
3. After ten (10) minutes, and in the same timed sequence, add 0.5 ml of AST (SGOT) Color Reagent, mix gently, and immediately return to 37°C heating bath for another ten (10) minutes.
4. After ten (10) minutes, add 2.0 ml of 0.1 N Hydrochloric acid and mix by inversion.
5. Set the wavelength of the spectrophotometer at 530 nm and zero the instrument with the Blank. Read and record the absorbance of all tubes. (Wavelength range: 500 – 550 nm)

NOTE: The final color developed in the reaction must be read within sixty (60) minutes.

CALCULATIONS

Use the absorbance reading of the calibrator and unknown(s) to calculate AST (SGOT).

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Calibrator}} \times \text{Conc of Calibrator (IU/L)}$$

= AST (SGOT) values of Unknown (IU/L)

Example:

Abs. (unknown) = 0.094

Abs. (calibrator) = 0.084

AST(SGOT) concentration of Calibrator = 38 IU/L

$$\frac{0.094}{0.084} \times 38 = 43 \text{ IU/L}$$

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established AST (SGOT) values may be used for quality control. The assigned value of the control material must be confirmed by the same methodology. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

LIMITATIONS

1. Endogenous pyruvate does not interfere with this method as it does with the dinitrophenylhydrazine method.
2. Bilirubin in concentrations of 5 mg/dl and upward can cause falsely elevated values. A serum blank can eliminate this false reading.
3. Erythrocytes contain approximately ten (10) times the normal concentration of transaminase found in serum.⁸ Hemolysis in the specimen must be avoided. If the specimen is lipemic or icteric, a serum blank should be run.

EXPECTED VALUES⁸

10 – 40 IU/L at 37 °C.

It is strongly recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

PERFORMANCE CHARACTERISTICS

1. Linearity: 500 IU/L.
2. Sensitivity: Based on an instrument resolution of 0.001 absorbance, the present procedure has a sensitivity of 0.5 IU/L.
3. Comparison: A comparison study between the present method with an available commercial product using the same identical method on twenty fresh serum samples and two commercial serum controls, ranging from 12 IU/L to 198 IU/L yielded a coefficient of 0.99 and a regression equation of $y = 1.02 x = 1.89$.
4. Precision studies:

Within run: Two commercial serum controls were assayed twenty times and the following within run precision was obtained.

	<u>Within Run</u>	
<u>Mean(IU/L)</u>	<u>S.D.</u>	<u>C.V.%</u>
44	3	6
174	4	2

Run-to-run: Two commercial serum controls were assayed for a period of thirty (30) days (duplicate for each level), the following run-to-run precision was obtained.

	<u>Run to Run</u>	
<u>Mean(IU/L)</u>	<u>S.D.</u>	<u>C.V.%</u>
44	4	9
187	17	9

REFERENCES

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