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

\[
\text{L-Aspartate + 2-Oxoglutarate} \rightarrow \text{Oxalacetate + L-Glutamate}
\]

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
AST(SGOT) Substrate : 33 mM Aspartic acid, 5 mM ketoglutaric acid, phosphate buffer pH 7.4.

AST(SGOT) Color Reagent: 0.25% w/v Diazonium salt preserved with formalin.

AST(SGOT) Calibrator: A lyophilized serum with AST(SGOT) value provided in each lot. Reconstitute with distilled water, let stand until dissolved and swirl to mix. Stable for five (5) days at 2 – 8°C after reconstitution. Aliquot into small portions and keep frozen.

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
All reagents are ready to use.

STORAGE AND STABILITY
Store AST(SGOT) Substrate, AST(SGOT) Color Reagent and AST(SGOT) Calibrator in refrigerator (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 serum. Reports indicate that AST(SGOT) in serum remains stable at 4°C for a minimum of 7 days. Hemolized 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 activity.⁶ Refer also to N.E. Saris for a list of references.⁷

QUALITY CONTROL
It is recommended that control 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 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.

EXAMPLE OF CALCULATION
Abs.(unknown) = 0.094
Abs.(calibrator) = 0.084
AST(SGOT) concentration of Calibrator = 38 IU/L
PROCEDURE LIMITATION
1. Endogenous pyruvate does not interfere with this method as it does with the dinitrophenyl-hydrazine 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. Hemolysis in the specimen must be avoided. If the specimen is lipemic or icteric, a serum blank should be run.
4. EXPECTED VALUES
10 – 40 IU/L at 37°C
It is strongly recommended that each laboratory establish its own normal range.

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 = 1.02x - 1.89.
4. Precision studies:
Within Run precision: Two commercial serum controls were assayed twenty times and the following Within Run precision was obtained.

<table>
<thead>
<tr>
<th>Mean (IU/L)</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>3</td>
<td>6%</td>
</tr>
<tr>
<td>174</td>
<td>4</td>
<td>2%</td>
</tr>
</tbody>
</table>

Run to Run precision: 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.

<table>
<thead>
<tr>
<th>Mean IU/L</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
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<td>44</td>
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<td>9%</td>
</tr>
<tr>
<td>187</td>
<td>17</td>
<td>9%</td>
</tr>
</tbody>
</table>

REFERENCES

Date Revised: 07/06