

AST (SGOT) Color

TEST PROCEDURE FOR SPECTROPHOTOMETER

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

Procedure Conditions

Wavelength	 540 nm
Temperature	37° C
Mode	Endpoint
Sample to Reagent Ratio	1:33

TEST PROCEDURE (1)

- A. Label reagent tubes, as "BLANK", "CONTROL" and "PATIENT".
- B. Incubate reagent tubes at 37° C for 5 minutes.
- C. Add 0.8 ml of SGOT reagent to each tube.
- D. Add 100 µl of normal control serum and to the "CONTROL" tube. Cap the tube and mix well by inversion.
- E. Add 100 µl of patient serum sample to the "PATIENT" tube. Cap the tube and mix well by inversion.
- F. Incubate reagent tubes at 37° C for 60 minutes.
- G. Add 0.5 ml of color developer A to each reagent tube. Cap the tubes and mix well by inversion.
- H. Let reagent tubes stand at room temperature for 20 minutes.
- I. Add 2 ml of color developer B to each reagent tube. Cap the tubes and mix well by inversion. Let tube stand at room temperature of 5 minutes.
- J. Wipe the reagent tubes clean with a lint-free tissue.
- K. Place the "BLANK" tube in the test well and adjust the photometer to zero absorbance.
- L. Place the "CONTROL" and "PATIENT" tubes in the test well and record the absorbance of the "CONTROL" and "PATIENT" samples.

Calculation Patient SGOT concentration =

$$\frac{\text{absorbance of "PATIENT" sample}}{\text{absorbance of "CONTROL" sample}} \times \text{X mean assay value of control}$$

EXPECTED VALUES Normal range: 8 - 40 U/L at 37° C

- NOTE:
1. a. Careful control of temperature and timing is essential for the accuracy and precision of test results.
 - b. The final reaction color is stable for 30 minutes.

INTENDED USE

These reagents are for the quantitative determination of Serum Glutamate-Oxalacetate Transaminase (SGOT) enzyme activity in serum.

SUMMARY AND EXPLANATION

Glutamate Oxalacetate Transaminase (GOT) is one of the amino transferase enzymes which catalyze the reversible reaction of amino acids and alpha-ketoglutaric acid by the transfer of the amino group. GOT is present in large amounts in heart, liver muscle and kidney tissue. Consequently the determination of serum GOT could serve as a valuable aid in different diagnoses.

In 1957 Reitman and Frankel (1) presented a colorimetric approach to measure GOT activity. An end product of the transamination reaction, oxalacetate, is reacted with dinitro phenylhydrazine (DNPH) to form the hydrazone complex. This hydrazone is reacted with an alkaline diluent to form a colored complex which can be measured quantitatively in a spectrophotometer or a colorimeter. The Biotron Diagnostics method is the modification of Reitman and Frankel.

TEST PRINCIPLE

The enzyme glutamate oxalacetate transaminase (GOT) catalyzes an exchange of an amino group of aspartate for an alpha-keto group of alpha ketoglutarate. The end products formed in this reaction are oxalacetate and glutamate.

The oxalacetate formed partially decomposes to pyruvate in a constant ratio under the condition of the test. Dinitrophenylhydrazine is added to form the hydrazones of the keto acids present. These hydrazones are sequentially reacted with sodium hydroxide to form a color which can be read by a spectrophotometer or colorimeter.

MATERIALS PROVIDED

SGOT (AST) Reagent 100 ml
Color developer A 60 ml
Color developer B 120 ml
Standard/Control/Calibrator

REAGENTS

For In Vitro Diagnostic use.

1. SGOT (AST) Reagent contains 0.5 ml phosphate buffer containing 0.02 M alphaketoglutaric acid, 0.2 M dl-aspartate and sodium azide as a preservative.
2. Color Developer A contains 0.003 M 2,4-dinitrophenylhydrazine, 1 M hydrochloric acid, preservative.
3. Color Developer B contains 1.6% sodium hydroxide.

CAUTION! Do not take these reagents internally or allow them to come in contact with the body.

STORAGE

Store reagents in refrigerator at 2-8° C. All reagents are stable till the expiration date stated on the label when stored in refrigerator.

ADDITIONAL MATERIALS REQUIRED

1. Blood analyzer, spectrophotometer, or colorimeter
2. Pipetting devices
3. Commercially available assayed control serum

SAMPLE AND PREPARATION

Freshly drawn serum sample.

EXPECTED VALUES

The normal range of SGOT is 8 to 40 U/L at 37° C.

The above range is intended as a guide. Each laboratory should establish its own normal range.

PERFORMANCE

1. Precision - The precision study was done by
 - (a) repetitive assay (N=23) of normal serum. The assay yielded a mean of 17 U/L and a standard deviation of 3 U/L and a coefficient of variation of 17.6%.
 - (b) 8 day reproducibility. A pool serum specimen yielded a mean of 14 U/L a standard deviation of 2 U/L and a coefficient of variation of 14.3%.
2. Accuracy - The accuracy study was done by running 28 specimens on Dade (trademark of Dade Diagnostics Inc.) SGOT kit and Biotron Diagnostics Kit on Starsar III (registered trademark of Gilford Instruments). The study yielded a regression equation of Biotron = 1.027 * reference method - 2.06 and a correlation of 0.91.

LIMITATIONS OF THE PROCEDURE

This procedure is linear from 0 to 120 U/L at 37° C.

QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used. Daily quality control must fall within 2 standard deviations of the established value. If correlation is not obtained and repetition of the assay excludes error in technique, the following steps should be taken:

1. Calibrate the instrument according to manufacturer's instructions.
2. Check the cleanliness of the reagent tube.
3. Check the expiration date of the reagent package.
4. Contact BiotronDiagnostics Technical Services Department in Indianapolis, IN.

REFERENCES

1. Reitman, S. and Frankel, S., American Journal of Clinical Pathology, 28:56-63, 1957.
2. Henry, R. J., Cannon, D. C. and Winkelman, J. W., "Clinical Chemistry, Principles and Technics," 2nd Ed., 1974, Harper and Row, New York, 884-889.
3. Kin Diagnostics Laboratory Data, Indianapolis, IN, 1981.