An Assay and Screening Procedure for Serum Glutamic Oxaloacetic Transaminase

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A clinical method for measuring serum GOT activity and its application as a screening test for GOT are described. Results for sera, compared by two popular methods, correlated well. This method may be suitable for routine use as a screening test in the blood bank.

Additional Keyphrases: spectrophotometry • citrate synthase • inter-method comparison

Serum glutamic-oxaloacetic transaminase (L-aspartate:2-oxoglutarate amino transferase, EC 2.6.1.1), an extensively studied enzyme, is measured in serum for differential diagnosis, prognosis, and the selection of bloods for transfusion. Thus, to prevent hepatitis infection after transfusion, the laboratory measures G0T activity in blood of donors. Enhanced activity indicates possible liver disorders. There also is need for a quick laboratory examination of serum G0T to assist in the diagnosis of acute myocardial infarction. These needs for a simple, accurate, and quick determination of G0T at the blood bank led us to develop the procedure described here.

G0T activity has been assayed by a number of methods in recent years, including colorimetric procedures in which the keto acid hydrazone (1) or keto acid tetrazolium salt complex (2) is formed, a continuous procedure for direct measure of oxaloacetate absorbancy (3), a spectrophotometric method in which G0T is coupled with malate dehydrogenase (4), and continuous colorimetry of G0T coupled with citrate synthase (5).

We have adapted the citrate synthase [citrate oxaloacetate lysase (CoA acetylating), EC 4.1.3.7] method (5) for clinical use.

Materials and Methods

Most of the methods and materials are unchanged from those previously described (5). Sera were obtained from the clinical laboratories at the Dallas Veterans Administration Hospital. All reagents are stable for at least a month.

(a) Citrate synthase–DTNB method. The method and principle were described previously (5). The assay depends upon the reactions

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\text{2-Oxoglutarate} + \text{aspartate} \xrightarrow{\text{GOT}} \text{glutamate} + \text{oxaloacetate}
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\text{Oxaloacetate} + \text{acetyl-S-CoA} \xrightarrow{\text{citrate synthase}} \text{citrate} + \text{CoASH}
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\text{CoASH} + \text{DTNB} \rightarrow \text{CoAS-TNB} + \text{TNB}
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The increase in absorption due to the TNB anion is measured at 412 nm. The assay mixture contains 0.2 ml of Tris–Cl buffer (1 mol/liter, pH 8.1), 0.2 ml of aspartate (0.2 mol/liter), 0.02 ml of acetyl CoA (0.01 mol/liter), 2 μl of citrate synthase solution (0.5 mg/ml), 0.1 ml of DTNB (1 mmol/liter), 0.01 ml of EDTA (0.1 mol/liter), an aliquot (0.05 to 0.1 ml) of serum, and water to make a final volume of 0.98 ml—all in a 1-cm cuvet. The final pH is 8.1. Under these conditions, a slow increase in absorbance occurs.
(blank reaction) because of the reaction of DTNB with serum proteins. This increase is linear after 10 min. Reactions were started by the addition of 20 µl of 2-oxoglutarate (0.1 mol/liter). A Gilford spectrophotometer was used to follow the increase of absorbance with time at 412 nm at 25°C. The molar absorptivity index (ε_M = 13,600 at pH 8.1) was used to translate the increase in absorbance into micromoles. The units of serum G0T activity are given in micromoles of CoASH produced per minute per liter of serum at 25°C (cs unit).

(b) Malate dehydrogenase-DPNH method. We have compared the present assay method with the widely accepted malate dehydrogenase-coupled method for the determination of serum G0T activity (4). The incubation was as described above, but oxalacetate was determined as it was formed in the presence of NADH and malate dehydrogenase by measuring the decrease in absorbance at 340 nm, due to NADH oxidation. The reaction mixture contained 0.2 ml of Tris-Cl buffer (1.0 mol/liter, pH 8.1), 0.2 ml of aspartate (0.2 mol/liter), 0.05 ml of malate dehydrogenase (0.5 mg/ml), 50 µl of DPNH (2.5 mg/ml), 20 µl of 2-oxoglutarate (0.1 mol/liter), and serum to a final volume of 1.0 ml—all in a 1.0-cm cuvet. Reactions were initiated with 2-oxoglutarate after a 5-min preincubation. The molar absorptivity value used was 6200 for DPNH. The serum G0T activity was expressed as micromoles of oxalacetate produced per minute per liter of serum (MDH unit).

(c) Clinical laboratory method. Serum transaminase activity was determined in the Veterans Administration Hospital chemistry laboratory at Dallas by use of the automated 12-channel serum analyzer (“SMA 12/30,” Technicon Corp., Tarrytown, N.Y. 10591), according to the manufacturer’s method. Values for G0T greater than 40 units are considered abnormal (LAB unit).

(d) Screening test for serum G0T activity. The reaction mixture was: Tris-Cl buffer (0.2 mol/liter, pH 8.1), aspartate (40 mmol/liter), 2-oxoglutarate (2 mmol/liter), acetyl-CoA (2 mmol/liter), EDTA (0.1 mmol/liter), and citrate synthase (1 µg/ml). Into the wells of a plastic tray or into small tubes, 0.5 ml of reaction mixture was pipetted, and one drop (about 0.03 ml) of sample serum or standard serum, the activity of which was previously determined by another method, was added. The contents were mixed by swirling, and kept at room temperature on a white paper. A yellow color (caused by TNB anion) develops with time. About 10 to 20 min later, samples and standards are compared.

(e) Alternative screening test. Reagent solution, 0.3 ml, containing 0.3 µmol of DTNB, 0.5 µmol of acetyl-CoA, 30 µg of citrate synthase, 50 µmol of potassium phosphate buffer (pH 7.5), and 3 µmol of 2-oxoglutarate, were transferred to the small cuvet made for the Bausch & Lomb “Spectronic 20” spectrophotometer (0.5-in. test tube, catalog no. 33-29-27), frozen, and lyophilized (sample reaction tube). A blank reaction tube was similarly prepared, but without 2-oxoglutarate. Tubes were kept in the freezer at -20°C before use. Buffer–substrate solution was made as 0.04 molar aspartate–1 mmolar EDTA–0.15 molar potassium phosphate buffer, pH 7.5. For assay of activity, 3.0 ml of buffer–substrate solution was added to both the reaction tube and blank tube, to dissolve the pellet in the tube, and kept at room temperature for 2 min. Serum, 0.15 ml, was added to both tubes, which were incubated for 10 min at 37°C. Absorbance was measured vs. blank tubes at zero time and after 10-min incubation, with a Spectronic 20. The standards were run as in the above procedure, without incubation and with use of a known amount of CoASH solution, or with incubation using reference serum instead of the sample serum.

Results and Discussion

Figure 1 shows the relationship, for serum, between l-aspartate and 2-oxoglutarate concentrations and reaction velocity as plotted by the Lineweaver–Burk method and using the citrate synthase–DTNB method. From these data the K_m for aspartate was calculated to be 9.5 × 10^{-3} mol/liter, that for 2-oxoglutarate, 9.0 × 10^{-4} mol/liter. The substrate concentration used for the method was then fixed at 40 mmol/liter for the aspartate and 2 mmol/liter for the keto acid.

![Fig. 1. K_m determination for l-aspartate and 2-oxoglutarate](image)

Activities (cs units) were expressed as µmol CoASH produced in 5 min/0.025 ml of a patient’s serum containing 500 LAB units. Activities were assayed with cs-DTNB method as described in method a. For the determination of K_m for 2-oxoglutarate the concentration of l-aspartate was 40 mmol/liter. For the determination of K_m for l-aspartate the concentration of 2-oxoglutarate was 2 mmol/liter.

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The values for the $K_m$'s are similar to those for the soluble type of G6P and indicate that the G6P originated from cytoplasm.

Plasma contains 133 μmol of sulphydryl per liter (θ). Thus, 50 μl of serum may have 6.75 to 17.5 nmol of sulphydryl groups. The reaction between the sulphydryl groups of serum and DTNB is complete only after 25 min of incubation (θ), and 50 μl of serum may cause an absorbance change of 0.09 to 0.24 at 412 nm. But the rate of absorbance increase at 412 nm is almost constant after 9 min preincubation, and the color of the blank is not as intense as calculated.

If the sera reflects the presence of jaundice, carotenemia, or anemia, the blank value and sample value may be greater, but this does not interfere with the assay.

Results by the new method were compared with those for the LAB unit and MDH unit (Figure 2). The results indicate that the cs method possesses sufficient sensitivity for the determination of serum G6P, and also that values for serum G6P greater than 9 units for the cs method and 10 units for the MDH method are to be considered abnormal.

The screening test, performed as described, is not suitable for yellow serum. Such colored serum can be assayed spectrophotometrically or colorimetrically, but the method described in the text under e is more accurate than that under d. Since large amounts of aspartate cannot be lyophilized with DTNB, it was necessary to use the two separate reagents for the reaction. If there is no incubator or colorimeter, the tubes can be incubated at room temperature and compared visually with the blank reaction tube and the reference serum. The amount of color developed is linearly related to the amount of enzyme used.

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References