Automated Procedure for Serum Aldolase Estimation

Fructose-1, 6-Diphosphatase and Fructose-1-Phosphatase

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An automated procedure for serum aldolase estimation is described. Using the same reagents, but different substrates, muscle and liver aldolase are differentiated. The procedure is based on the manual method of Bruns (1). A close correlation between manual and automated methods is shown.

Serum aldolase estimation is one of the principal diagnostic tests used in the elucidation of muscle disease (2-6). In myocardial infarction (7), liver disease (8), and carcinomatosis (9), the serum aldolase estimation is a useful ancillary aid to diagnosis. The usefulness of the test has been enhanced since the differentiation between muscle and liver aldolase by use of two substrates—fructose-1,6-diphosphate (for muscle aldolase) and fructose-1-phosphate (for liver aldolase)—was described (10).

The automated method described below is based on the manual test reported by Bruns (1).

Materials and Method

Reagents

Substrates

Fructose-1,6-diphosphate (0.05 M, pH 7.4) Dissolve 0.2 gm. of the sodium salt (Sigma grade*) in 10 ml. of Tris buffer (below). This may be kept frozen until required.

Fructose-1-phosphate (0.05 M, pH 7.4) Prepare as above, but use 0.15 gm. of the sodium salt.

The barium salts, which are cheaper, may be used for the preparation of the above substrates after precipitation of the barium, as described by Friedman and Lapan (11). In this case the amount of salt used is

*Sigma Chemical Company, St. Louis, Mo.
varied to compensate for the different molecular weight and for the degree of purity of the preparation used. It should be noted that the barium salt of fructose-1,6-diphosphate contains traces of fructose-1-phosphate. The result of this is a slight shift in the muscle aldolase: liver aldolase ratio which, in general, for clinical purposes can be ignored.

Tris buffer, pH 7.4 Add 103.5 ml of 0.2 N HCl to 125 ml of 0.2 M Tris (24.2 gm of tris(hydroxymethyl)aminomethane per liter), and make up to 500 ml. Add 0.3 ml of Triton X-405 (wetting agent).

Hydrazine (0.56 M, pH 7.4) Add 14.56 gm. hydrazine sulfate to 100 ml water. Adjust to pH 7.4 with NaOH; the hydrazine will then dissolve. Make up to 200 ml with water.

Iodoacetic acid, 0.66 mM Dissolve 40 mg of iodoacetic acid in 100 ml of distilled water and add 200 ml of Tris buffer. This reagent should be prepared fresh daily.

2,4-Dinitrophenyl hydrazine stock solution Dissolve 1 gm in 1 liter of 2 N HCl.

2,4-Dinitrophenyl hydrazine working solution Add 20 ml of methanol to 40 ml of stock solution and make up to 100 ml.

Recipient solution Dissolve 5 gm of anhydrous sodium carbonate in 1 liter of distilled water. Add 0.6 ml of Triton X-405.

Sodium hydroxide, 0.8 N (CO₂ free)

Standard Stock solution: Dissolve 200 mg of dihydroxyacetone in 100 ml of distilled water. This stock solution of 2 mg/ml must be placed in the refrigerator for 48–72 hr. to complete depolymerization. Standards are prepared by dilution of stock standard with distilled water, so that a range of 0.1–1.5 mg/ml is obtained.

The reagents used are essentially those of the manual method except for changes necessitated by the automated procedure. (Consequently, in an emergency the reagents are at hand for use in the manual method.) Tris buffer has been substituted for the collidine buffer, and the final concentrations of 2,4-dinitrophenyl hydrazine and iodoacetic acid have been altered to overcome the problem of “noise” in recording.

Procedure

A flow diagram of the manifold and other components is shown in Fig. 1. The specimens are run at a speed of 50 per hour using a 2:1, sample:water wash ratio. Although a 540 mμ filter has been used, any filter over the range of 505–550 mμ is satisfactory.

Time is saved if serum blanks are run first. The substrate lead is left in water for the blank run. When this is completed, aspiration of the required substrate is started. In practice, the microtube for the sub-
substrate is kept as short as possible to minimize the time taken to reach the serum-substrate mixing point. Since only 0.1 ml. of substrate is used per test, the substrate can be held in a Bijou bottle which is placed on the manifold plate.

![Flow diagram for automated determination of aldolase](image)

**Fig. 1.** Flow diagram for automated determination of aldolase. Sampling 50/hr.; cam 2/1; flow cell 15 mm.; filter 540 m.

To run a standard curve, the patient’s serum is replaced by dilutions of the stock standard covering the range 0.1–1.5 mg./ml. The aldolase units equivalent to the dihydroxyacetone standards may be derived from the formula:

\[
\text{Dihydroxyacetone (mg./ml.)} \times \frac{60}{\text{incub. time (min.)}} \times 60 \text{ (corr. factor)} = \text{aldolase (DHA units)}
\]

The aldolase units thus derived coincide with the “dihydroxyacetone units” of Friedman and Lapan (11). The incubation time is calculated by introduction of a dye into the buffer line and then timing the progress of the dye from Point A to Point B (Fig. 1). Incubation time is in
Table 1. Comparison of Results Between Automated and Manual Method

<table>
<thead>
<tr>
<th>Specimen &amp; type*</th>
<th>Fructose-1,6-diphosphatase† (DHA units)</th>
<th>Fructose-1-phosphatase† (DHA units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manual</td>
<td>Automated</td>
</tr>
<tr>
<td>1 (N)</td>
<td>2.5</td>
<td>4</td>
</tr>
<tr>
<td>2 (N)</td>
<td>12.5</td>
<td>12</td>
</tr>
<tr>
<td>3 (N)</td>
<td>13.5</td>
<td>10</td>
</tr>
<tr>
<td>4 (MI)</td>
<td>140</td>
<td>150</td>
</tr>
<tr>
<td>5 (MI)</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>6 (MI)</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>7 (IH)</td>
<td>134</td>
<td>125</td>
</tr>
<tr>
<td>8 (IH)</td>
<td>84</td>
<td>78</td>
</tr>
<tr>
<td>9 (IH)</td>
<td>130</td>
<td>129</td>
</tr>
<tr>
<td>10 (CIH)</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>11 (MD)</td>
<td>116</td>
<td>122</td>
</tr>
<tr>
<td>12 (MD)</td>
<td>110</td>
<td>111</td>
</tr>
<tr>
<td>13 (MD)</td>
<td>96</td>
<td>105</td>
</tr>
<tr>
<td>14 (MD)</td>
<td>55</td>
<td>60</td>
</tr>
</tbody>
</table>

* N, normal; MI, myocardial infarction; IH, infectious hepatitis; CIH, convalescent infectious hepatitis; MD, muscular dystrophy.
† Normal values accepted for manual and automated procedure: fructose-1,6-diphosphatase <20 units; fructose-1-phosphatase <4 units.
minutes and the seconds are converted to the decimal fraction of a minute (e.g., 16 min. 24 sec. equals 16.4 min.). The correction factor has been experimentally derived and is the same for all AutoAnalyzers.

Alternatively, a pooled serum with known high aldolase value may be used for preparation of standards. Serum from patients with infectious hepatitis (to ensure high liver aldolase) should be included in the serum pool. If infectious hepatitis serum is unavailable, normal serum to which liver homogenate has been added can be used. (Approximately 10 ml. of the soluble component of a 1:10 w/v rabbit liver homogenate added to 90 ml. of human serum will bring both aldolase activities into the desired range.) The two aldolase values are estimated by the manual method; the serum is divided into small amounts and frozen (−20°C) until required. The aldolase activity will remain unchanged for at least 6 months.

A recorder tracing of an actual test run of 12 different sera is shown in Fig. 2. Also illustrated are examples of serum and dihydroxyacetone standard curves.

As seen from Table 1 the correlation between the manual and the automated method is good and within the range of experimental error.

References