Automated Determination of Anti-Streptolysin O Antibodies by a Kinetic Hemolytic Method

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Received 12 June 1984/Accepted 20 August 1984

An automated method which uses oxidized Streptolysin O and determines the anti-Streptolysin O titer in whole blood without dilution of the sample is described. The addition of a reducing agent starts the hemolysis at an initial rate which is inversely proportional to the anti-Streptolysin O titer of the sample. An instrument (Taso-matic), designed to automatically execute all of the procedures, was used. Comparison between the described method and the classical hemolytic method gave the following linear regression slope y = 1.06x – 13 (r = 0.974). Precision of the method at two different anti-Streptolysin O titers (200 and 500 IU), expressed as relative standard deviation, was 12.1 and 4.7% in the between-run procedure and 10.1 and 4.9% in the within-run procedure, respectively.

A serological test which determines the anti-Streptolysin O (ASO) titer is important for the diagnosis of some streptococcal infections (1–9). The first method for determining the ASO titer was described by Todd (7) and successively modified to improve its practicality (1, 6, 9). Ricci and colleagues (5) described a manual microtechnique, using different amounts of oxidized Streptolysin O (SO), 2-mercaptoethanol as the reducing agent, and whole blood as the sample. We describe a modification of this technique that is suitable for automation. The method uses oxidized SO (3, 4, 8) and 1-cysteine as the reducing agent; 1-cysteine can be considered to be the hemolysis starting reagent. We could determine the ASO titer in blood samples without dilution by measuring the initial rate of hemolysis. A new instrument (Taso-matic), which automatically performs all steps after the sample has been added to the oxidized SO in a photometric cuvette, has been used. A microprocessor and C-MOS technology render this instrument easy to use, and a built-in photometer allows as many as 18 determinations to be made simultaneously.

MATERIALS AND METHODS

Oxidized SO. Oxidized SO was obtained by culture of Streptococcus pyogenes group A (supplied by Centro Diagnostico Senese, Siena-Italy). Oxidation with hydrogen peroxide was carried out by the method of Ricci and colleagues (5). The working solution containing 2 combining units of SO per ml in isotonic saline was prepared and titrated with the International Standard Serum for ASO (obtained from the Danish State Serum Institat of Copenhagen). The working solution remains stable for up to 24 h at 4°C or for 1 year at −20°C.

Reducing agent. The reducing agent was a solution of 14 mmol of 1-cysteine per liter in isotonic saline. This solution remains stable for up to 24 h at 4°C.

Samples. Blood was collected from patients hospitalized for various diseases. Samples for the presented method were drawn in K3-EDTA for the counting procedures and without an anticoagulant to obtain serum for the classical reference method. Only fresh samples were used. Sera were decomplemented at 56°C for 30 min.

Artificial blood samples at known titer. Human sera titrated by the classical hemolytic method (6) and the resulting 200 and 500 IU were collected. A 0.55-ml volume of each serum was mixed with 0.45 ml of human erythrocytes (group O and Rh negative), previously washed in isotonic saline and centrifuged at 2,000 rpm for 10 min.

Instrumentation and other materials. Taso-matic and cuvette stirrers were manufactured and supplied by DIESSE Diagnostica Senese s.r.l., Monteriggioni, Italy. Disposable cuvetttes with a 1.0-cm light path were supplied by Polymed s.r.l., Florence, Italy. SMI positive displacement micropipets were from American Hospital Supply Corp. Emeryville, Calif.

ASO titer determination. A 2.5-ml volume of oxidized SO (2 combination units per ml) and 15 µl of the blood sample were dispensed into a disposable cuvette equipped with one stirrer. Each cuvette corresponding to a sample was inserted into the numbered holder of Taso-matic. One vial of reducing agent was also inserted into the proper holder on the instrument. Taso-matic was programmed according to the instruction manual of the manufacturer. Two programs were available: RANGE1 (200 to 650 IU, with 50-IU step) for routine and screening, and RANGE2 (650 to 2000 IU, with 150-IU step) for samples with more than 650 IU in RANGE1. The instrument automatically executes the complete analysis cycle, which consists of 15 min of incubation at 30°C to allow the antigen-antibody reaction, dispensing 0.5 ml of the reducing agent for each sample, absorbance measurements to determine the initial rate of hemolysis, data processing, and printing the results in international units, with sample identification number. The entire working cycle is completed within 30 min.

RESULTS

A total of 591 samples were tested by the described method (y) and by the manual hemolytic method (x) (6). Figure 1 shows the scattergram of the results. A linear regression slope, y = 1.06x – 13, resulted, with a correlation coefficient of r = 0.974. Precision studies of the between-run
procedure and within-run procedure were made by using the artificial blood samples with ASO titers of 200 and 500 IU (Table 1).

**DISCUSSION**

Our data indicate that the described method improves the procedure for quantitative ASO titer determination. Manual operations are reduced, and hemolysis reading is no longer made visually. Photometric readings make the method objective and precise. Precision reaches unusual levels for a serological method, whereas accuracy is comparable to that of the manual reference method. Furthermore, the kinetic mode eliminates any sample or SO dilution. Using the automatic instrument, we can run more samples simultaneously while obtaining the printed results within 30 min. L-

Cysteine was used as the reducing agent instead of 2-mercaptoethanol as in the previously described method (5) because there is less undesirable odor and it is less expensive at the concentrations used. Among the 591 tested samples, 48 had hematocrit between 30 and 40%, but the difference between ASO titers obtained by the described method and by the reference method never exceeded 100 IU. This may be explained by considering the fact that the samples with a low hematocrit value cause a lower erythrocyte concentration in the reaction mixture than do the samples with a normal hematocrit value, with a consequent higher initial rate of hemolysis and the tendency to give false low titers. In contrast in these same samples, the ratio between plasma and cell volume is higher; therefore, with the increase in plasma volume, there are more antibodies in the reaction mixture, with a consequent increase in ASO titer. The two phenomena probably tend to compensate one another, and the results obtained by the automated method do not substantially differ from those obtained by the reference method.

**LITERATURE CITED**


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**TABLE 1. Within run and between run precision**

<table>
<thead>
<tr>
<th>Artificial sample (IU)</th>
<th>Data measuring precision</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (IU)</td>
<td>SD (IU)</td>
</tr>
<tr>
<td>Within run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>211</td>
<td>21.4</td>
</tr>
<tr>
<td>500</td>
<td>525</td>
<td>25.7</td>
</tr>
<tr>
<td>Between run</td>
<td></td>
<td></td>
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<td>221</td>
<td>26.7</td>
</tr>
<tr>
<td>500</td>
<td>514</td>
<td>24.4</td>
</tr>
</tbody>
</table>

* RSD, Relative standard deviation.