Standardized Procedure for Determination of Staphylocoagulase

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Received for publication 28 January 1969

A simple standardized procedure for the determination of staphylocoagulase, based on thrombin clotting time measurement with euglobulin, has been developed. A standard euglobulin solution containing 0.25% of clottable protein, 0.01 M \( \varepsilon \)-aminocaproic acid, and 20 units of heparin per ml is employed. One unit of staphylocoagulase is defined as the amount of the enzyme which clots this standard euglobulin solution in 25 sec.

Various methods of staphylocoagulase detection and measurement have been reported (1, 2). These, however, are not sufficiently quantitative. Recently, two additional procedures have been described (6, 7). Both employ fibrinogen and coagulase-reacting factor as substrate. The nephelometric method (7) is laborious, and, in both procedures, measurement of the staphylocoagulase activity is unsatisfactory at high enzyme concentration because of the lack of proportion between staphylocoagulase and the coagulase-reacting factor.

The purpose of the present investigation was to develop a simple and standard method for the determination of staphylocoagulase. This was accomplished by using euglobulin as a source of both fibrinogen and coagulase-reacting factor.

MATERIALS AND METHODS

Plasma. Human blood was added to 3.8% sodium citrate solution and centrifuged at 3,000 rev/min for 20 min.

Substrates. A fraction of human plasma euglobulin was prepared by diluting citrated plasma with distilled water 1:20 and adjusting the pH to 5.3 with 1% acetic acid. After standing at 4°C for 20 min, the precipitate was centrifuged at 2,000 rev/min for 5 min at room temperature and dissolved to one-half of the initial volume of plasma in Palitsch buffer at pH 7.4 containing 0.01 M \( \varepsilon \)-aminocaproic acid. The resulting euglobulin solution contains 0.25% of thrombin clottable protein. This solution was stored at −20°C with 20 units of heparin added per ml for the prevention of spontaneous thrombin clotting. Heparin was without detectable effect on the determination of staphylocoagulase activity.

Fibrinogen was prepared by the method of Kekwick (4).

Clottable protein measurement. The standard method of Quick (5) was used for clottable protein measurement.

Thrombin. A freeze-dried product of Parke, Davis and Co., Detroit, Mich., was used.

Staphylocoagulase. A freeze-dried sample of staphylocoagulase was purified according to the method of Jeljaszewicz (3).

Heparin. Heparin was obtained from POLFA, Warsaw.

\( \varepsilon \)-Aminocaproic acid. Crystalline \( \varepsilon \)-aminocaproic acid was received from Fluka, Switzerland.

Preparation of thrombin standard curve. The standard curve must be run with freshly prepared euglobulin solution without heparin. For testing the thrombin clotting time, 0.4 ml of the standard euglobulin solution, 0.3 ml of Palitsch buffer, and 0.1 ml of thrombin solution were used. Dilutions were made within the range of 0.1 to 2.0 NIH units of thrombin per ml. The thrombin solution was prepared ex tempore. All manipulations were carried out in siliconized glass. The clotting time of the mixture was determined in a water bath at 37°C. The curve was prepared by plotting on logarithmic paper the reciprocal of the thrombin time in seconds against the thrombin units. Comparative curves were prepared by using, instead of the standard euglobulin solution, 0.4 ml of plasma or 0.4 ml of plasma diluted 1:40 with Palitsch buffer containing 0.25% of fibrinogen.

RESULTS

The clotting times of the three different substrates with thrombin at various concentrations are presented in Fig. 1. A straight-line relationship between the clotting time and the thrombin concentration was obtained only when the euglobulin solution was used as a substrate. The clotting times and the staphylocoagulase units read from three standard curves (Fig. 1) for the three staphylocoagulase concentrations are given in Table 1.

The closest relationship between the concentration of staphylocoagulase added and the units...
TABLE 1. Standard curves for the determination of staphylocoagulase by using three different substrates

<table>
<thead>
<tr>
<th>Staphylocoagulase concn</th>
<th>Euglobulin</th>
<th>Fibrinogen</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting time</td>
<td>Units</td>
<td>Clotting time</td>
<td>Units</td>
</tr>
<tr>
<td>mg/ml</td>
<td>sec</td>
<td>mg/ml</td>
<td>sec</td>
</tr>
<tr>
<td>0.05</td>
<td>72</td>
<td>10</td>
<td>0.16</td>
</tr>
<tr>
<td>0.10</td>
<td>42</td>
<td>0.30</td>
<td>42</td>
</tr>
<tr>
<td>0.20</td>
<td>28</td>
<td>0.80</td>
<td>37</td>
</tr>
</tbody>
</table>

determined was obtained when reciprocals of the clotting times were read from the standard euglobulin solution curve.

To standardize a staphylocoagulase preparation, the following procedure is proposed.

Reagents. Reagents include: (i) a known solution of staphylocoagulase; (ii) a standard euglobulin solution containing exactly 0.25% of clottable protein, 0.1% e-aminocaproic acid, and 20 units of heparin per ml; and (iii) Palitsch buffer, pH 7.4, prepared by mixing 0.05 M sodium borate (19.108 g of Na₂B₄O₇·10H₂O per liter of distilled water) with 0.2 M boric acid-salt solution (12.404 g of H₃BO₃ and 2.925 g of NaCl per liter of distilled water) until pH 7.4 is obtained. Approximately 1.2 parts of borate solution to 8.8 parts of boric acid-salt solution is required.

Test system. An 0.4-ml amount of the standard euglobulin solution, 0.2 ml of Palitsch buffer, and 0.2 ml of known staphylocoagulase solution (undiluted and diluted 1:2, 1:4, and 1:8) are required for the test system.

Procedure. A mixture of euglobulin solution and Palitsch buffer is preincubated for 1 min at 37°C, 0.2 ml of a staphylocoagulase solution is added, and the clotting time is recorded.

Reading of final results. By using the reciprocals of the clotting times obtained with the four staphylocoagulase dilutions, the thrombin units are read from the standard curves. The average mean values of the four staphylocoagulase dilutions (adjusted for dilution) are taken as the basis for calculating the staphylocoagulase units.

It is proposed that: one unit of staphylocoagulase is the amount of enzyme that clots a standard euglobulin solution containing 0.25% of clottable protein, 0.01 M e-aminocaproic acid, and 20 units of heparin per ml in 25 sec.

DISCUSSION

Our method for determining staphylocoagulase is based on a comparison of the clotting times of a standard euglobulin solution with known amounts of thrombin units. The euglobulin fraction of plasma has a substantial advantage over whole plasma or fibrinogen and the coagulase-reacting factor. When plasma is used, the clotting times at low concentrations of thrombin are usually markedly prolonged because of the presence of plasma antithrombin. On the other hand, this effect is not noted with staphylocoagulase, since this enzyme is not affected by the thrombin inhibitors. The use of plasma as a substrate may, therefore, lead to serious error.

Systems with fibrinogen also have a disadvantage. When a high concentration of staphylocoagulase is tested, the clotting time may be unproportionally long, owing to a lack of sufficient amount of coagulase-reacting factor.

All these difficulties are avoided by using euglobulins as the substrate. The coagulase-reacting factor is present in excess and antithrombins are absent. Once prepared, the euglobulin solution can be stored at —20°C, provided heparin is added to prevent spontaneous clotting. With the thrombin standard curve previously prepared, it is possible to measure the activity of a given staphylocoagulase preparation with a single determination of the euglobulin clotting time.

ACKNOWLEDGMENT

This investigation was supported by research grant CDC-LP-3 from the National Communicable Disease Center, U.S. Public Health Service.

LITERATURE CITED


