A Serum Reference Standard for Automated Total Protein and Albumin Procedures

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Preparation of a stable pool of concentrated serum, suitable for use as a reference standard in automated total protein and albumin procedures, is described.

In spite of the advocacy, from time to time, of purified serum albumin as a reference standard in protein estimations, many analysts prefer to use pooled sera for this purpose. Such a pool can serve as a reference standard for both total protein and albumin determinations. A difficulty arises, however, with automated procedures; a pool of serum collected in the laboratory usually does not have a sufficiently high total protein and albumin content to permit a check on linearity above the lower region of the normal range. With manual methods it is simple to extend the range of standards upward by increasing the volume of standard solution taken for the test, but with automated procedures it is usually necessary to use standards which themselves cover the required range. Concentration by dialysis of the large volumes of serum required is inconvenient and time consuming.

A suitably concentrated, stable standard may be prepared easily from a pool of unused patient sera by treatment with dried polyacrylamide gel, its use originally suggested by Curtin (1), who found that no measurable loss or denaturation of protein resulted. We used Lyphogel, a commercially available preparation (Gelman Instrument Co., P.O. Box 1448, Ann Arbor, Mich. 48106; Hawksley & Sons Ltd., 12 Peter Road, Lancing, Sussex, U.K.).

One liter of the pooled serum is allowed to stand refrigerated overnight with about 5 g of Lyphogel. The concentrated serum is then filtered and distributed among a number of tubes or ampoules and stored at -20°C. Total protein is determined by the Kjeldahl method, and albumin by the same method after fractionation with Na2SO4 (26 g/100 ml). In each case, the protein is precipitated with tungstic acid before digestion.

By use of a large pool, occasional variations associated with a few abnormal sera—e.g., possible anomalies in biuret chromogenic power or in salting-out behavior—are apparently swamped (Table 1).

<table>
<thead>
<tr>
<th>Pool</th>
<th>Total protein</th>
<th>Albumin</th>
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<tbody>
<tr>
<td></td>
<td>AutoAnalyzer (biuret method)</td>
<td>Kjeldahl (protein (\times 6.25)) g/100 ml</td>
</tr>
<tr>
<td>A*</td>
<td>7.85</td>
<td>8.05</td>
</tr>
<tr>
<td>B*</td>
<td>9.8</td>
<td>9.95</td>
</tr>
<tr>
<td>N</td>
<td>7.1</td>
<td>7.25</td>
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</tbody>
</table>

* Concentrated as described in text. In all cases, the standard was a concentrated pool of serum collected in the laboratory and analyzed as indicated in the text.

Reference standards prepared and stored this way are stable for at least five months. Immediately before use, a tube of the frozen serum is thawed and a range of dilutions is prepared.

It has recently been reported (3) that Lyphogel may be recovered by stirring with several changes of water, removing the gel pellets by filtration, and drying them at 65°C, thereby considerably reducing the cost of this product. Alternatively, dried acrylamide gel may be prepared in the laboratory as described by Curtin (1).

References


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