Letters to the Editor

Determination of Serum Total Iron-binding Capacity

The paper by Leggate and Crooks (1972) on the problems in quality control of total iron-binding capacity (TIBC), determined by the magnesium carbonate method, has prompted us to report our experience in determining TIBC, using the method of Ramsay (1957) with that of Young and Hicks (1965) on the Technicon AutoAnalyzer I.

Initially our performance in the Wellcome Group quality control scheme was poor as can be seen from table I. Similarly poor results were obtained with other commercial quality control sera, our values often being as much as 100 μg above the stated figure.

It was noticed that the more acceptable results coincided with the introduction of fresh batches of magnesium carbonate and it was thought that the magnesium carbonate might be deteriorating on exposure to air. Atmospheric moisture was a possible cause of this deterioration through increasing the degree of hydration of the magnesium carbonate, so it was decided to heat the magnesium carbonate before use to drive off excess water.

Preliminary observations, using commercial sera, were encouraging. With Hyland special control serum, which had a stated value of 370 μg/100 ml (acceptable range 330-410 μg/100 ml), we obtained a result of 450 μg/100 ml when using the unheated magnesium carbonate and 345 μg/100 ml with the heated magnesium carbonate. With Behringwerke human control serum, of stated value 399 μg/100 ml (range 369-429 μg/100 ml), we obtained results of 465 μg/100 ml with unheated, and 435 μg/100 ml with heated, magnesium carbonate.

A further experiment was performed in which two batches of horse serum were analysed, one with unheated magnesium carbonate and one with magnesium carbonate heated at 100°C before use. There were 15 samples in each batch. The batch analysed using unheated magnesium carbonate gave a mean value of 347 μg/100 ml (SD ± 9.3), whereas that using the heated had a mean of 332 μg/100 ml (SD ± 9.5). The difference between the means was statistically highly significant (t = 6.5, p < 0.001).

We decided, therefore, to keep the magnesium carbonate in a draught-free oven at 100°C and remove sufficient for cooling just before each batch of sera was analysed.

Since we adopted this procedure we have obtained the improved performance figures for the Wellcome scheme, shown in table II.

These data show that the standard technique for determining TIBC (Ramsay, 1957; Young and Hicks, 1965) can give an acceptable degree of accuracy and precision provided that the magnesium carbonate has been stored previously at 100°C.

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References


Problems in the Determination of Serum Total Iron-binding Capacity

Good precision has not been achieved with methods for the determination of total iron-binding capacity (TIBC), although serum iron can be measured with high precision. It has been interestingly shown recently that the apparent TIBCs of some control sera are dependent on the amount of basic magnesium carbonate added to remove excess iron used to saturate the transferrin (Leggate and Crooks, 1972). The sera which showed this dependence all had a pH greater than 8.5. It has been clearly demonstrated, however, that regulation of pH at all stages of the procedure was very important when basic magnesium carbonate was used to remove excess iron (Williams and Conrad, 1972). It has also been suggested that magnesium carbonate does remove some bound iron from transferrin (Koepe, 1965).

One alternative method of removing excess added iron is by addition of anion-exchange resin, as in the AutoAnalyzer method (Technicon method file N-629).
When ion-exchange resin is used, then the TIBC is virtually independent of the amount of resin added, when more than about 100 mg of resin is used. This independence has been found with both human and bovine sera, and is not dependent on pH. Serum TIBC is slightly affected, however, by the state of the resin used (Lehmann and Kaplan, 1971).

However, the apparent TIBC, when ion-exchange resin is used, is markedly affected by the initial pH of the serum. This has been demonstrated with both bovine and human sera, either fresh or freeze-dried; the serum pH was adjusted by drop-wise addition of N hydrochloric acid or N sodium hydroxide (Table).

To overcome this problem, titration of each serum to the same initial pH and ionometry were attempted but were rather impractical for routine use. A simple satisfactory solution, which in our laboratory has led to improved precision and to improved inter-laboratory comparison, has been to add barbitone buffer pH 7-5 to each serum before saturating the transferrin with iron, as opposed to the final pH adjustment in the standard AutoAnalyzer methodology.

This factor, and other factors such as that studied by Leggate and Crooks (1972), can contribute markedly to the large inter-laboratory variation found in regional and national quality control schemes as many commercial freeze-dried and in house control sera have a high pH, and again emphasizes that caution should be exercised in the interpretation of quality control data.

<table>
<thead>
<tr>
<th>Serum</th>
<th>pH</th>
<th>Apparent TIBC (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh human</td>
<td>6-5</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td>8-4</td>
<td>383</td>
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<td></td>
<td>9-5</td>
<td>461</td>
</tr>
<tr>
<td>Fresh bovine</td>
<td>7-0</td>
<td>394</td>
</tr>
<tr>
<td></td>
<td>8-0</td>
<td>551</td>
</tr>
<tr>
<td></td>
<td>10-0</td>
<td>596</td>
</tr>
<tr>
<td>Freeze-dried human</td>
<td>7-5</td>
<td>338</td>
</tr>
<tr>
<td></td>
<td>8-4</td>
<td>416</td>
</tr>
<tr>
<td>Freeze-dried bovine</td>
<td>7-5</td>
<td>608</td>
</tr>
<tr>
<td></td>
<td>8-9</td>
<td>652</td>
</tr>
</tbody>
</table>

Table Effect of initial pH on the apparent TIBC

Standardization of Clinical Enzyme Assays

The publication of a definitive report on the standardization of clinical enzyme assays is a consumption devoutly to be wished by all clinical biochemists but it is doubtful if any such report would have their unreserved approval. However, the report by Wilkinson, Baron, Moss, and Walker (1972) can elicit little sympathetic response from any.

The first prerequisite of any standardization of clinical enzyme assays is to decide upon a standard temperature. This was speculated on by the authors, who 'first considered 37° as the most suitable' and then sought evidence against it, only finding comfort in a putative glucose-6-phosphate dehydrogenase instability which incidentally is contrary to our own experience (King, 1972). However, this apparently was sufficient to make for a decision in favour of 25°C for estimating transaminase activities. Certainly we cannot find any argument advanced by the authors in favour of 25°C 'there is often a lag phase of 2 to 5 min' is considered an advantage. How temperature control is readily accomplished by using tap water as an external cooling unit baffles us and our credulity is inordinately exercised to believe that the reason why 30°C has not been generally adopted in this country is because 25°C can be maintained by this device 'for most of the year'. Presumably for that part of the year when it is not practicable one must patiently await the return of more favourable climatic conditions.

The same logic is applied to the reactant concentrations. The substrate concentrations employed by Henry, Chiamori, Golub, and Berkman (1960) at 32°C, as modified for alanine transaminase by Arvan and Coyle (1969) at an unstated temperature, are proposed for use at 25°C, even though it is acknowledged that the L-alanine (and hence 2-oxoglutarate) concentration is suboptimal. We have found no solubility problems with 810 mM L-alanine nor have Boehringer or Merck in their commercial test kits. This problem has been artificially created by the use of separate buffer and substrate solutions, aided by the unnecessary suggestion that D,L-alanine can be used.

On 50 sera, using the Boehringer 'optimized' test combinations (Bergmeyer and Bernt, 1963), we have found very similar temperature correction factors between 25°C and 37°C, that is, 2-18 and 1-93, with coefficients of variation of 4-45 to 6-30 and 7-8 (King, 1973a) for aspartate and alanine transaminase respectively. These figures indicate that considerably more than caution should be exercised in using temperature conversion factors. Our normal ranges for aspartate and alanine transaminase by optimized assay at 37°C are 13-42 mU/ml (females 10%, lower) and 11-55 mU/ml (females 25% lower) with respective coefficients of variation of 2-0 to 5-0% and 1-5 to 5-9% depending upon the level of activity and whether the determinations were within-batch or between-batch (King, 1973b). With the lower activities obtained at 25°C the quality of our results was poorer but the normal range of activities approximated that given in the Boehringer literature and considerably higher than that obtained by Wilkinson and colleagues (1972). We can therefore emphatically state that the techniques recommended do not 'give maximal activities' as claimed by the Working Party.

Again we are perplexed to understand why the lack of specificity in the recommended transaminase assays brought about by the presence of glutamate dehydrogenase should only be mentioned in a footnote concerning liver extracts. The same source of error is present in sera where hepatic necrosis is present but is readily overcome by using lactate and

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**References**


