Present day practice

Problems in quality control in determinations of serum total iron-binding capacity by the magnesium carbonate method

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The precision of a semiautomated method for total iron-binding capacity (TIBC) of serum seemed to be much poorer than expected. Preliminary investigation suggested that the poor precision was associated with the control serum and not the patient's serum. This paper describes experiments which were carried out to investigate this phenomenon.

Method (Ramsay, 1957)

Serum was mixed with a solution of ferric chloride to saturate the transferrin with iron and the excess iron removed by the addition of basic magnesium carbonate. After centrifugation, the iron in the supernatant was determined by a modification (Babson and Kleinman, 1967) of the Young and Hicks method for serum iron (1965).

Efficacy of Different Grades of Basic Magnesium Carbonate

Basic magnesium carbonate is obtainable in three grades—'light', 'heavy', and 'for chromatographic adsorption analysis'. Ramsay, in his original method (1957), recommended the use of the 'light' grade while Jordan and Podmore (1963) used the grade 'for chromatographic adsorption analysis'. The three grades show some difference in their capacity to remove unbound iron from human serum which has been treated with a solution of ferric chloride. In particular the grade 'for chromatographic adsorption analysis' gave consistently higher results for TIBC (by about 50 $\mu\text{g}/100\text{ ml}$) and this may be a contributory factor to the variations in results obtained by different laboratories.

Amount of Magnesium Carbonate

A more important factor which can contribute to the

Received for publication 25 April 1972.

variation between laboratories and within a laboratory is the quantity of magnesium carbonate added. It is common practice to add approximately 200 mg magnesium carbonate by filling a test tube to a level which has been previously shown to correspond to 200 mg. In our experience this can lead to errors of $\pm 50\text{ mg}$ in the amount added. With serum from patients the amount of magnesium carbonate is not critical but with some commercial control sera the amount of iron removed from the serum/ferric chloride mixture is dependent on the amount of magnesium carbonate added (Table I). This effect is

Serum	Apparent TIBC ($\mu\text{g}/100\text{ ml}$)		
	Magnesium Carbonate		
	100 mg	200 mg	300 mg
Human	410	395	385
Control	460	320	240

Table I Apparent TIBC of serum when different amounts of magnesium carbonate are added

not limited to any particular brand of control serum nor is the effect present to the same degree in different batches of the same brand. Most of our experiments have been carried out with Wellcontrol unassayed serum but similar effects have been found with Versatol, Chemonitor, Hyland, and a freeze-dried serum of bovine origin supplied in our regional quality control programme. We were not able to demonstrate the effect in a batch of fresh unadulterated bovine serum nor in fresh unadulterated pig serum. Sera which demonstrated dependence of the TIBC on the amount of magnesium carbonate all had a pH greater than 8.5 (measured at 37°C). It was found that altering the pH to less than 8.5 caused the apparent TIBC of the control serum to become

pH	Apparent TIBC ($\mu\text{g}/100\text{ ml}$)		
	Magnesium Carbonate		
	100 mg	200 mg	300 mg
9.7	500	340	260
8.8	340	270	230
8.4	250	240	240
7.1	240	240	240

Table II Effect of initial pH of commercial control serum on the apparent TIBC with different amounts of magnesium carbonate

independent of the amount of magnesium carbonate (Table II). On the other hand, increasing the pH of human serum to 8.9 did not make the apparent TIBC dependent on the amount of magnesium carbonate (Table III).

pH	Apparent TIBC ($\mu\text{g}/100\text{ ml}$)		
	Magnesium Carbonate		
	100 mg	200 mg	300 mg
8.9	330	320	320
8.1	330	320	320
6.8	330	320	320

Table III Effect of the initial pH of human serum on apparent TIBC with different amounts of magnesium carbonate

Discussion

These experiments demonstrated that the apparent TIBC of some control sera is dependent on the amount of magnesium carbonate added, whereas the apparent TIBC of patients' sera is virtually independent of the amount of magnesium carbonate used. Since it is common practice to add an approximate 200 mg of magnesium carbonate in this method, errors are incurred in the measurement of the TIBC of control sera which are not incurred when measuring the TIBC of patients' serum. This means that the quality control data are not an accurate reflection of the analytical performance of the laboratory.

From Table II it can be seen that if the pH is altered to less than 8.5 the apparent TIBC of control serum becomes independent of the amount of magnesium carbonate and this provides a simple method of ensuring that the quality control serum is in fact measuring analytical precision. Table II also shows that if 300 mg of magnesium carbonate is added instead of 200 mg the result is less dependent on the initial pH and this is another possible modification. However, in view of our findings that under practical conditions the amount of magnesium carbonate added may vary by $\pm 50\text{ mg}$ we would not recommend this. The most satisfactory solution would be for the manufacturers of the control serum to ensure that the final pH is less than 8.5.

It is likely that there are problems yet to be recognized in the use of control sera for other estimations, and caution should be exercised in the interpretation of quality control data, particularly in interlaboratory schemes. Unless variations due to the control serum are eliminated the data do not reflect accurately the performance of the laboratories.

References

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