Present day practice

Erroneously high results in an AutoAnalyzer method for the determination of iron on patients' sera

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As manual methods for estimating the concentration of iron in serum are replaced by automated methods it is not always appreciated that the results may not be identical. Babson and Kleinman (1967), in their experiments with 'serum protein solutions', found that the AutoAnalyzer procedure of Young and Hicks (1965) gave iron results which did not agree with a manual method (Peters, Giovannello, Apt, and Ross, 1956) and were erroneously high. They showed that the discrepancy was due to a Donnan equilibrium effect on the dialysis rate of the ferrous iron, an essential step in the AutoAnalyzer procedure. Because of this effect, the protein present in the test 'serum solutions' led to a greater dialysis rate than in the standard iron solutions. After the addition of 5% NaCl to the diluent reagent (1% ascorbic acid in 0.1 M HCl), they found that the automated procedure gave iron assays which agreed with the manual method and gave good recovery of iron added to 'serum protein solutions'.

This modification of the automated procedure is now used routinely by most laboratories. However, when patients' sera are analysed, although the

Iron Added ($\mu\text{g}/100\text{ ml}$)	Iron Recovered ($\mu\text{g}/100\text{ ml}$)							
	Manual Method		AutoAnalyzer Methods					
			Unmodified		Modified with 5% NaCl		Modified with 10% NaCl	
Experiment								
1 and 2	1	2	1	2	1	2	1	2
49	48	49	58	62	55	53	52	52
98	98	99	123	126	106	105	103	106
147	148	145	190	197	160	163	162	164
196	193	199	250	259	215	216	214	217

Table Recovery of iron added to serum calculated after the estimation of the metal by four different methods in two experiments

Fig. 1 Comparison of serum iron results from a manual and an AutoAnalyzer method. The regression line (—) lies about 10% above the line which would have been obtained had the results been identical (.....).

modification of Babson and Kleinman greatly improves the results, a correction is still necessary to get complete agreement with the manual methods. This is demonstrated by the results of two recovery experiments in which known amounts of iron added as ammonium ferric sulphate solution to iron deficient serum were estimated in duplicate by (a) the manual method of Trinder (1956), (b) the unmodified AutoAnalyzer method of Young and Hicks (1965), (c) as (b) but with 5% NaCl added to the diluent reagent (modification of Babson and Kleinman

(1967) and (d) as (b) but with 10% NaCl added to the diluent reagent.

This finding is substantiated by the results from 67 different sera analysed in duplicate by the manual and the modified AutoAnalyzer methods (Fig. 1). Although there is good correlation between the two methods (correlation coefficient $r = 0.995$), the results given by the latter method are higher (regression coefficient $b = 1.11$).

An attempt to improve further the modified AutoAnalyzer method by increasing the concentration of NaCl from 5 to 10% was unsuccessful (Table). The use of sera with known iron content to replace the standard iron solutions is the ideal way of correcting the discrepancy. However, this is not always practicable and it is suggested that a correction factor (-10%) should be applied to results obtained by the AutoAnalyzer procedure.

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Technical methods

A simple concentration method for the detection of parasitic ova and cysts in faeces

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During the course of a survey of intestinal parasitic ova and cysts in recently arrived immigrant children carried out by this laboratory, (Thompson, Hutchison, and Johnston, 1972), it rapidly became obvious that a method was required which would fit easily into the routine of a busy laboratory. Such a method was required to yield as many positives for helminth ova and protozoal cysts as possible on the single specimen submitted, and to be technically simple and not time consuming. The method evolved makes use of concentration by centrifugation combined with the addition of a dye mixture: this gives (1) good contrast on microscopy of the wet preparations and (2) tolerates the addition of Lugol's iodine for the confirmation of protozoal cysts, since the combined dye mixture is stable.

Method

A portion of faeces about the size of a pea is emulsified with a wooden stick in a tube of peptone broth (5 ml). With a large-bore (approximately 2 mm external diameter) Pasteur pipette the supernatant is transferred to a centrifuge tube. Two drops (approximately 0.05 ml) of the stain mixture, consisting of equal parts of 10% (w/v) aqueous Nigrosin and 1% (w/v) aqueous Alcian Blue, is added, together with 4 to 5 drops of concentrated formalin solution (40 vol %). The tube is centrifuged at 3 000 revs/min for five minutes in the MSE Minor. Most of the supernatant is then discarded into Lysol, leaving a volume of fluid equal to that of the button of deposit. With a Pasteur pipette the mixture is gently homogenized and 'wet preparations' are made. Scanning for ova and cysts is carried out using the 16 mm objective with a daylight filter for three to five minutes.

To confirm the morphology of protozoal cysts,

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