Intrinsic factor antibody tests

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SUMMARY The sensitivity of methods to detect antibodies to intrinsic factor was assessed. Five sera of known antibody content were tested in 31 laboratories and 30 sera from patients with pernicious anaemia were tested in one laboratory. Five non-commercial methods and two kits for type I antibodies and one non-commercial method for types I and II antibodies are in current use. Differences in sensitivity of the non-commercial methods for type I antibodies related more to the antigen:antibody ratio in the test system than to the method itself. A radioimmune assay for types I and II antibodies showed the best sensitivity but that of an enzyme linked immunosorbent assay (ELISA) method was poor

Type I antibodies (IFA) to intrinsic factor, which block the binding of cobalamin to intrinsic factor, have been recorded in the 31% to 76% of patients with pernicious anaemia.¹ This large variation may be due to case selection or to differences in technique used, or both. Because the detection of IFA may avoid the need for further investigation in the diagnosis of pernicious anaemia we compared the sensitivity of some of the methods in current use, including two which detect type II antibodies (inhibitors of the attachment of intrinsic factor to the ileal mucosa) at the same time. Tests for type II antibodies alone are not often used because the antibodies are thought to be less common than type I antibodies, though this may not be so.²

Material and methods

Sera were collected from patients with pernicious anaemia and from a healthy control. These were coded and sent to laboratories which undertake IFA testing and which had agreed to test samples for us. Two sera from patients with pernicious anaemia containing, respectively, 6 and 2 units IFA/ml were issued first and on a second occasion, patients' serum containing 10 units IFA/ml together with this serum diluted by a factor of 8 and 10 in the normal serum, and the normal serum were sent.

The methods used by participants were those of Ardeman and Chanarin,³ of Gottlieb *et al.*,⁴ and of Ghazi,⁵ using gastric juice, commercial intrinsic factor,

or the reagents from a commercial B12 kit (Becton Dickinson; BD); and the IFA kit of Corning and the IFbab kit of Diagnostic Products Corporation UK(DPC). Two other methods used were the radioimmune assay with ¹²⁵I-labelled intrinsic factor of Conn⁶ and the Melisa kit, a micro/ELISA technique, of Walker Laboratories Ltd. Both these are said to detect types I and II antibodies simultaneously.

Thirty sera from other patients with pernicious anaemia and 20 sera from surgical patients were collected for a comparative study in our laboratory of the methods of the IFbab and Melisa kits. For the Ardeman and Chanarin method 1.25 ng intrinsic factor and 300 µl serum were used and for Ghazi method 3 ng intrinsic factor and 200 µl serum per tube were used.

Results

Thirty one and 29 laboratories returned results for the two issues, respectively. The results with the five patient sera are shown in table 1, according to method used. That of Conn—used by two participants—gave the highest detection rate. The four other non-commercial methods gave a similar incidence of positive results. All laboratories recorded the normal serum as negative.

In table 2 the results from the second issue are related to the relative proportion of antigen (ng intrinsic factor) to antibody (ml serum) in the test system when these were known. There is a clear inverse correlation between the antigen:antibody ratio and the ability to detect weak antibodies. The results with

Table 1 Inter-laboratory issues: results with five sera containing IFA

	Non-commercial methods						Kits		
	Ardeman and Chanarin	Ghazi	Gottlieb <i>et al</i>	Conn	BD	Not stated	IFA	IFbab	Melisa
No of sera tested	22	5	13	10	30	16	12	33	8
No positive	11	2	5	7	10	1	2	14	0
Positive (%)	50	40	39	70	33	6	17	42	0

commercial kits and commercial kit reagents are shown in the same table.

The results with the 30 patient sera are shown in table 3 in which the findings obtained with the Ardeman and Chanarin method are compared with the others. This and the Ghazi method gave identical results. The Conn method produced the most positive results though it missed three found positive by the Ardeman and Chanarin method. The fewest positive results were obtained with the Melisa though it recorded one positive which was negative by all the others. The 20 sera from surgical patients were negative by all methods except for two by Melisa.

Discussion

The non-commercial methods, other than the Conn method, gave an overall similar incidence of positive results in the inter-laboratory tests. Laboratories using the same technique, however, differed considerably in their rates of antibody detection. None use the volumes of gastric juice and serum originally proposed for the Ardeman and Chanarin and Gottlieb *et al* methods. Unfortunately, the concentration of intrinsic factor needed is not given for these methods, nor for that of Ghazi. All use normal gastric juice, but after pentagastrin stimulation this may have an intrinsic factor content ranging from 15 to 115 units/ml. The volumes used by the participants varied from 50 to 500 μ l and the serum volumes from 20 to 300 μ l. The results obtained here show that it is the relative

concentrations of the reagents rather than the method which determines the detection rate for type I IFA. There is an inverse correlation between the antigen:antibody ratio and the sensitivity of the test system. The sensitivity of the Gottlieb *et al* method has been improved by a considerable reduction in the amount of intrinsic factor⁷ and the techniques^{8,9} with the highest detection rates require proportions of antigen to antibody around unity.

If excess antigen is present a small reduction in B12 binding will not be seen and even a strong antibody could be missed. With low intrinsic factor concentrations, however, any difference between the unsaturated transcobalamin concentrations of control and test sera may become obtrusive, although this was not seen with the Ghazi method. With 300 μ l volumes the usual test sera may have a binding capacity of 0.1 ng more than a control of pooled normal serum. With 1 ng or more of intrinsic factor this difference is not important but with lesser amounts the non-specific binding has to be controlled, as in the Ghazi method, or neutralised by prior treatment of the sera. The results in table 3 show that with 4.2 units intrinsic factor/ml serum the Ardeman and Chanarin method gave the same results as the Ghazi method with 10 units intrinsic factor/serum (the proportion expected with the average gastric juice). Thus there is no advantage in using the Ghazi method with its additional control tubes.

We therefore recommend the Ardeman and Chanarin method for routine use with 1.25 ng intrinsic

Table 2 Second inter-laboratory issue: results related to proportion of intrinsic factor to serum in system

Serum			Non-commercial methods (n = 12)	Other methods (n = 16)	
Patient serum	Diluted by a factor of 8	Diluted by a factor of 10	(ng IF: ml serum)	Reagent proportion not available	
+	+	+	4:1 (Ardeman and Chanarin)	5:1 (Ardeman and Chanarin)	Conn, IFbab
+	+	Indeterminate	8:1 (Gottlieb <i>et al</i>)		
+	+	Indeterminate	50:1 (Gottlieb <i>et al</i>)		
+	Indeterminate	Indeterminate			Conn, BD
+	Indeterminate	—	20:1 (Ardeman and Chanarin)		
+	—	—	25:1 (Ardeman and Chanarin)	33:1 (Ardeman and Chanarin)	BD(2) Corning IFbab (4)
			140:1 (Ghazi)		
Indeterminate	—	—			BD
—	—	—	25:1 (Gottlieb <i>et al</i>)	50:1 (Ardeman and Chanarin, 2)	BD (2) Corning
			(Unknown)		

Figures in parentheses indicate number of laboratories when more than one.

Table 3 Results with 30 sera with pernicious anaemia
Comparison of results by the Ardeman and Chanarin method
with four other methods

		Ghazi		IFbab		Conn		Melisa	
		+	-	+	-	+	-	+	-
Ardeman and Chanarin	+	17	0	16	1	14	3	11	6
	-	0	13	0	13	5	8	1	12

factor and 300 μ l serum per tube. These proportions detected the weakest antibodies in the inter-laboratory issues. With these volumes an incubation time for the antigen:antibody reaction extended to 30 minutes is preferable. We also suggest a change in the calculation of the binding inhibition with the non-commercial type I methods. The formulae customarily used require the difference between negative control (= 100% intrinsic factor activity) and the test serum counts to be calculated. At low concentrations of inhibition this difference is small and the total error large. The ratio of control to test counts reduces this error and improves the ability to detect low concentrations of IFA.

The greater incidence of positive results with the Conn method are probably because it detects type II antibodies also. If this is the case it implies that the Conn method is less sensitive to type I antibodies as it missed three. We did, however, have technical problems with the method. The 125 I label readily came off the intrinsic factor on storage, necessitating the addition of more intrinsic factor to the system (and removal of free isotope by charcoal), with subsequent reduction in sensitivity. If type II antibodies are as common as type I antibodies a sensitive method which

detects both should be at an advantage: The Conn method was a case in point; the Melisa was not.

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