Evaluation of a commercial radioassay for the simultaneous estimation of vitamin B₁₂ and folate, with subsequent derivation of the normal reference range

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SUMMARY A commercial assay kit method for the simultaneous estimation of vitamin B₁₂ and folate concentrations has been evaluated. Values derived for folate by a microbiological assay and vitamin B₁₂ by a verified radioassay showed good correlation with the investigated method. The clinical sensitivity of the assay for detecting deficient concentrations of vitamin B₁₂ and folate was comparable to that of the non-commercial methods and other more definitive clinical procedures. Establishment of reference ranges, based on accepted statistical criteria, are discussed and such ranges are contrasted with those proposed by the manufacturer. The kit method is time and labour saving compared with the non-commercial methods.

For many years microbiological assays have been used as reference methods for measuring vitamin B₁₂ and folate concentrations in blood. These methods were established as sensitive indicators of deficiency, but are subject to problems of antibiotic and antineoplastic drug interference and are also both time and labour intensive.

Radioisotope dilution assays have recently gained wide acceptance as alternative procedures, and their increased use can be attributed largely to an ease of use coupled with negligible drug interference. Some radioisotope dilution assays, however, experienced major specificity and interference problems, especially for vitamin B₁₂ measurements. Other shortcomings centre on inadequate evaluations and determination of appropriate reference ranges.¹⁻³ A recent extension of radioisotope dilution assays has been the development of a method for the simultaneous determination of both vitamin B₁₂ and folate. This latter method has the potential to be both cost and labour effective and may be a solution to the problems previously outlined. Certain aspects of commercial kits using these newer techniques

have been evaluated elsewhere.⁴ Information is limited to the assay of vitamin B₁₂ and includes the kit evaluated in this study.

Definitive reference methods for vitamin B₁₂ and folate are not available;⁵ most radioisotope dilution assays are judged against microbiological methods. Under these restrictions the true bias of such assays cannot be evaluated.

The purpose of this study was to address certain questions and problems associated with a commercial dual radioisotope dilution assay method. The evaluation centres on an assessment of precision and bias, the latter based on correlation with non-commercial methods and overall recovery of added vitamin. An assessment of the clinical sensitivity of the commercial method is based on correlation studies with assay values by non-commercial methods and results from more definitive clinical procedures. The non-commercial methods included a microbiological assay for folic acid⁶ and a radioisotope dilution assay for vitamin B₁₂.⁷ Reference ranges for the kit were established by adherence to accepted statistical criteria.⁸ Several other factors such as antibiotic interference in the non-commercial method for folate and the economics in terms of cost, time, and labour associated with each method are also discussed.

Material and methods

MATERIAL

Kits

Micromedic B₁₂/Folate Combostat II kits (Micromedic Systems Inc, Horsham, PA, USA) and control sera were obtained from Australian Diagnostics Corporation Pty Ltd (Victoria, Australia). Each kit was capable of assaying 100 tubes.

The principle of the assay kit is based on a "no boil solid phase" approach. This involves the release of vitamins from their respective binders in blood or serum by an alkaline solution containing sodium hydroxide and potassium cyanide at pH 12–13. At this pH it is claimed that intrinsic factor binding antibodies and the endogenous vitamin B₁₂ binding proteins of the type found in some patients with the myeloproliferative disorders are denatured. If not adequately denatured, both factors would give rise to spuriously high results by radioisotope dilution assay.

The binding reagent contains purified hog intrinsic factor which is free of "R" protein. The binding by cobinamide and other vitamin B₁₂ analogues is thus claimed to be low in this assay. Also contained in the same preparation is β -lactoglobulin, a folate binder sensitive to common monoglutamate species. Both binding proteins are covalently bound to insoluble cellulose particles, thus allowing easy separation of free from bound fractions by simple centrifugation. The binding step takes place at pH 9.3.

Quality control sera

Control material was not supplied with each kit but was purchased separately from the manufacturer. Known as the Micromedic B₁₂/Folate Combostat II Control Set, the material consisted of three freeze dried preparations (low, normal, and high) each containing varying amounts of dl-N5-methyltetrahydrofolic acid and cyanocobalamin in a protein base. In addition, two serum pools containing various concentrations of vitamin B₁₂ and folate were prepared from a large number of patient samples. These were used as quality control samples in the long term.

Patients' samples

Fifty seven samples were used to generate comparison data between assay methods for both serum vitamin B₁₂ and folate, while 42 samples provided data for red cell folate comparison. The specimens were obtained from our hospital patients and were derived from various medical faculties. Specimens were chosen on the basis of assay values obtained with our non-commercial methods.

In a further study the results of 46 patient samples assayed by kit method were correlated with results from selected bone marrow aspiration and ⁵⁷Co B₁₂ plasma absorption and urinary excretion tests. Fifteen of these patients had abnormal ⁵⁷CoB₁₂ plasma absorption and urinary excretion results. The remaining 31 patients had bone marrow comments indicating frank megaloblastic change, or suggesting B₁₂/folate assays or showing features consistent with, but not necessarily diagnostic of, megaloblastosis. These features included dyserythropoiesis, nuclear-cytoplasmic asynchrony of the erythroblasts, and the presence of hypersegmented neutrophils or giant metamyelocytes. This was a retrospective study and encompassed results accumulated for a period exceeding five months.

Reference samples

Samples for the normal population were collected from 28 hospital staff and 99 volunteer blood donors at the time of donation. These comprised 62 men and 65 women, with an age range of 20–64 years. All donors were subjected to a routine complete blood picture (Coulter S, Coulter Electronics Ltd, England) before being accepted for the study.

Sample handling

All specimens were allowed to stand at room temperature for about 4 h. After clot retraction, the specimens were centrifuged and the serum was removed and immediately frozen.

Reference and patient samples were stored at –20°C. Samples used in the comparison of assay methods were assayed within one month or less of initial routine assay. All samples were preservative free.

Equipment

All dispensing operations were carried out using Oxford Liquid Handling Instruments (Lancer, USA). Repetitive volume additions used in the normal range study were achieved using an Eppendorf Multipette (Eppendorf Geratebau, West Germany). Radioactive counting was carried out on the dual channel Packard Autogamma 5210 gamma counter (Packard Instrument Company Inc, USA). Calculation of results, determination of the normal reference ranges, and most statistical operations were performed by a program written for an Apple II microcomputer.

METHODS

Analytical methods

Each kit was used according to the manufacturer's instructions with two basic exceptions. Firstly, the assay tubes used were of dimensions 16mm ×

100mm in order to suit our gamma counter. Secondly, for convenience, the red cell folate procedure used a 1/20 dilution of whole blood in a 1% ascorbic acid solution, which also contained a trace of 10% Tween 80. This was the recommended procedure for the non-commercial method,⁶ with the determination by kit method performed on this same preparation. The use of Tween 80 was discontinued after the comparison study as the manufacturer did not require its use.

The non-commercial method for vitamin B₁₂ determination was that of Tibbling.⁷ Verification of this method against the microbiological assay using *Euglena gracilis* has been reported elsewhere.⁹ The non-commercial method for serum folate and red cell folate determinations was the microbiological method essentially as described by Waters and Molin⁶ using a chloramphenicol resistant strain of *Lactobacillus casei*. All determinations were performed in duplicate.

Quality control studies

Short term quality control was monitored from the results of the kit control sera assayed in each run. Extended precision was ascertained after completion of the evaluation study. This was based on the assay of serum pools prepared for this purpose, which were assayed in duplicate in each run. Within run precision was based on the standard deviation (SD) of duplicates within assay for all assays. Between assay precision was based on the SD between the means for all assays. Information for 51 assays was collected using eight different kit batches.

Dilution studies

Limited data were generated from patient samples with raised vitamin concentrations. Neat assay values were compared with those values obtained when samples were diluted in zero standard at 1/10 dilution.

Recovery experiments

Two serum pools were spiked with dl-N5-methyltetrahydrofolic acid and cyanocobalamin of known concentrations derived from the kit standards. The serum integrity of each spiked pool was kept relatively intact by the use of one volume of spike to four volumes of serum. Determinations were performed in quadruplicate. Recovery was calculated by subtracting the base pool value from the spiked pool assay value, expressing the difference as a percentage of the amount of vitamin added.

Comparison studies

The results generated from assay by both kit and non-commercial methods were regressed using the

Deming regression model of analysis.¹⁰

Estimation of normal ranges

Both parametric and non-parametric (percentile) methods were used to evaluate the normal ranges for the kit method.⁸ Parametric estimation entailed prior transformation of the data, using logarithmic or square root functions, until minima were achieved for skewness and kurtosis.

Correlation at the deficient level

This was ascertained in two ways: firstly, by comparing the rate of agreement between the non-commercial method and kit method at the deficient level; and secondly, by comparing the results obtained by the kit assay method with more definitive clinical procedures on selected patients. An assay value was considered deficient by either method if it was below the defined 95% reference range (see table 3). For the Combostat II kit the deficient cut off was that determined by parametric analysis of the normal range. For the non-commercial methods these values were 148 pmol/l for vitamin B₁₂, 6.8 nmol/l for folic acid, and 363 nmol/l for red cell folate.

Assay data reduction

The assay standard curves were calculated by computer program using a log-logit transformation of the dose response variables. Fig. 1 shows a typical computer print out of assay information.

Assessment of cost, assay time, and labour requirements

This was based on 1800 requests for combined vitamin B₁₂, folic acid, and red cell folate determinations on each patient sample as expected by this laboratory over a period of 12 months. The assessment also takes into account a repeat determination rate of about 10% and the assay of two quality control specimens per assay.

As the kit allows the determination of only one folate at a time, the assessment of every red cell folate entails "wastage" of one vitamin B₁₂ determination. This extra cost is unavoidable with this method of determination and accordingly has been taken into consideration.

Results

TECHNICAL EVALUATION

Precision studies

Both within run and between run precision figures are presented in Table 1. The mean within run coefficients of variation (CV) were 5.2% for vitamin B₁₂ and 4.2% for folic acid. Similarly, mean between

**** FOLATE STANDARD CURVE

SLOPE = -1.011 INTERCEPT = 1.277

DATE OF ASSAY: 27/02/84 MAXIMUM BINDING (%) : 33.7

ACTUAL	PREDICTED	% DIFF
1	1	0
1	1	0
2	2	0
2	2	0
4	4.2	5
4	4.2	5
10	9.7	-3.01
10	9	-10.01
20	20	0
20	20.1	.5

***** UNKNOWN

#	DUPL #1	DUPL #2	MEAN	% DIFF
1	11.9	12.1	12	.6
2	18.7	18	18.4	1.8
3	3.5	3.5	3.5	.5
4	4.3	4.3	4.3	.4
5	2.8	3	2.9	3.7
6	4.5	4.2	4.3	3
7	14.8	15.5	15.2	2.3
8	3.6	3.5	3.5	.8
9	54	56.4	55.2	2.2
10	3.5	3.7	3.6	2.2
11	7.5	7.2	7.4	1.9
12	2.3	2.3	2.3	1.9

Fig. 1 Typical computer printout of assay information for folic acid. Results are expressed in ng/ml. nmol/l = ng/ml $\times$ 2.27.

Evaluation of Combostat II dual radioassay

Table 1 Precision data for the Combostat II assay kit

	Mean	Within run		Between run	
		SD	CV%	SD	CV%
Vitamin B ₁₂ (pmol/l)					
Level 1	104.2	6.7	6.5	19.1	18.3
2	332.0	12.7	3.9	26.7	8.3
Folic acid (nmol/l)					
Level 1	3.6	0.18	4.9	0.34	9.6
2	26.6	0.89	3.4	2.68	10.1

SD = standard deviation.
CV = coefficient of variation.
n = 102.

run CVs were 13.3% and 9.9%, respectively, with a higher variation at the lower vitamin B₁₂ concentration. Some of the latter imprecision may be attributed to inter-kit variation rather than inter-assay precision.

Dilution studies

Raised serum vitamin concentrations showed no clinical discrepancy with neat values when assayed at a 1/10 dilution.

Recovery

Results of the spiked recovery of added vitamin are presented in Table 2. The mean recoveries were 102.8% and 96.3%, respectively, for vitamin B₁₂ and folic acid.

Correlation between methods

Fig. 2 shows the results of regression analysis. Test results which exceeded the upper measurable limit of the method were excluded. Any test result which deviated from the regression line by more than 4 SDs was also omitted. An underlying cause for any other major discrepancy was then further investigated. As a consequence five of seven patients compared were eliminated because of antibiotic interference in the microbiological assay.

DETERMINATION OF REFERENCE RANGES

Frequency distribution coefficients and data transformation

All three vitamin determinations before transformation showed positive skewness, with red cell folate also showing kurtosis. In a normally distributed population skewness is zero and kurtosis has a value

Table 3 Effect of statistical method on the 95% normal reference range (n = 127)

	Parametric	Non-parametric	Manufacturer
Vitamin B ₁₂ (pmol/l)	172-557	167-565	163-554
Folic acid (nmol/l)	5.9-21.8	5.5-22.9	3.9-35.2
Red cell folate (nmol/l)	397-1126	390-1333	477-2225

of 3.0. If the kurtosis value exceeds 3, the distribution has longer tails than a normal distribution with the same standard deviation. Red cell folate showed the greatest statistical deviation from the normal distribution. After mathematical transformation of data, all three determinations had skewness and kurtosis coefficients which were not significantly different from the normal distribution.

Reference ranges

Age, sex, and packed cell volume did not affect the reference ranges for any of the vitamins. Non-parametric analysis was applied to the non-transformed values as the distributions showed a deviation from normality. Parametric estimates were applied to the transformed values as these data indicated normal distributions after transformation. The resulting effect of statistical method is shown in Table 3.

CORRELATION AT THE DEFICIENT LEVEL

Table 4 shows the correlation between the non-commercial methods and Combostat II in the deficient area for both vitamins. Three discrepancies were detected between methods for vitamin B₁₂ determination. Two of these discrepancies were not considered significant as comparison concerned assay values that had deviated marginally from one of the deficient cut off values. Both cases had normal haemoglobin concentrations and mean cell volumes. In the other discrepancy (normal/low) a raised mean cell volume favoured the determination by kit method.

In contrast, folic acid determination showed greater variation, with seven discrepant values. In all but one case comparison concerned values that had once again deviated marginally from one of the deficient cut off values. One of these patients was anaemic, but none had a raised mean cell volume. The

Table 2 Recovery of added vitamin

	Pool	Base value	Amount added	Amount recovered	% recovery
Vitamin B ₁₂ (pmol/l)	1	128	148	163	110.0
	2	304	593	559	94.4
Folic acid (nmol/l)	1	27.2	4.5	4.5	100
	2	3.4	18.2	16.8	92.5

Fig. 2 Regression analysis of patient sample results derived by Combostat II and non-commercial methods.

significant discrepancy (low/normal) had a normal haemoglobin concentration and mean cell volume, again favouring the kit method.

Correlation of the red cell folate values revealed

Table 4 Detection rates between methods at the deficient level* (Non-commercial method v Combostat II)

	Agreement		Discrepancies	
	Low/Low		Normal/Low	Low/Normal
Vitamin B ₁₂	13		3 (1)	0
Folic acid	16		1	6 (1)
Red cell folate	10		2	1

*Determinations with values below the defined 95% reference range for either method. Numbers in parentheses represent discrepancies considered significant (see results for explanation).

three discrepancies, none of which was significant for reasons similar to those already described. No patient had a low haemoglobin concentration or raised mean cell volume.

In general, the haematological data favoured the determination by kit method. Unfortunately, only five patients in this comparison were followed up with bone marrow aspiration or ⁵⁷Co B₁₂ plasma absorption and urinary excretion tests. Of four patients in agreement for low vitamin B₁₂ concentration, three were subsequently diagnosed as having pernicious anaemia or malabsorption of vitamin B₁₂ and the other had probable dietary deficiency. The fifth patient was deficient in serum folate by both methods, with the bone marrow showing features diagnostic of acute myeloid leukaemia.

Table 5 Correlation of vitamin B₁₂ concentrations by Combostat II with ⁵⁷Co B₁₂ plasma absorption and urinary excretion results.

Case no	Haemoglobin concentration (g/dl)	Mean cell volume (fl)	8h ⁵⁷ Co B ₁₂ plasma absorption	24h ⁵⁷ Co B ₁₂ urinary excretion	Conclusion or final diagnosis	Vitamin B ₁₂ concentration (pmol/l)	Folate concentration (nmol/l)	Red cell folate concentration (nmol/l)
1	11.5	75	Ab*	Ab*	PA or MA	118	N	N
2	6.9	118	Ab†	Ab†	PA	59	N	N
3	9.4	90	Ab†	Ab†	PA	155	N	N
4	12.0	104	Ab†	Ab†	PA	111	N	N
5	14.1	90	Ab‡	Ab‡	MA	78	N	N
6	6.9	90	Ab†	Ab†	PA	159	N	N
7	12.5	107	Ab‡	Ab‡	MA	48	N	N
8	9.4	92	Ab‡	N	Delayed absorption	87	N	N
9	12.4	86	Ab*	Ab*	PA or MA	113	N	N
10	14.5	74	N	N	Probable dietary deficiency	130	N	N

N = normal.

Ab = abnormal

NA = information not available.

PA = pernicious anaemia.

MA = malabsorption.

*Results of repeat test with intrinsic factor not available.

†Corrected with intrinsic factor.

‡Did not correct with intrinsic factor.

The results of an extensive clinical correlation are presented in Tables 5 and 6. Table 5 shows the correlation of assay results by kit method with haemoglobin concentration and mean cell volume, together with ⁵⁷Co B₁₂ plasma absorption and urinary excretion values. No bone marrow information was available for these patients. Similar information on further cases with available bone marrow information is presented in Table 6.

Of 31 patients with bone marrow information suggesting deficiency as outlined in the methods, 15 were deficient or borderline for any one or more than one of the vitamin B₁₂, folate, and red cell folate determinations. No patient showing frank megaloblastosis in the bone marrow examination had a normal result by the kit method. Of the 15 patients with abnormal ⁵⁷Co B₁₂ plasma absorption and urinary excretion results, 15 were deficient for vitamin B₁₂, which represents 100% agreement.

ASSESSMENT OF COSTS, ASSAY TIME, AND LABOUR

The cost of reagents and assay tubes of a combined vitamin B₁₂, folic acid, and red cell folate request, with each determination performed in duplicate, is about \$10.84 (£7.34) for the kit method. The figure may be less if the kits are purchased on a contract basis, but it is much higher than the cost of the non-commercial methods of \$2.10 (£1.42). In our laboratory, however, the non-commercial methods require two technicians whereas only one technician is needed for the kit method. This results in man-

power savings of about 41.5 h a week. The dual radioisotope dilution assay therefore allows an extra labour resource for the laboratory or the initial hiring of fewer staff to perform the assays.

Discussion

In order to evaluate the Micromedic B₁₂/Folate Combostat II kit we examined the performance of the kit in relation to certain common criteria, including methods established in the area of predictive and definitive clinical assessments.

The results of assay precision show that the Combostat II kit was generally reliable and reproducible in an extended study. The inter-kit batch variation at the lower vitamin B₁₂ concentration may reflect a problem with reagents or manufacturer quality control that may need to be further investigated. Average between and within batch CVs compare favourably with figures obtained for the same kit in another study⁴ and for other kits¹¹ and inter-laboratory evaluations.* No discrepancies were noted for raised values on sample dilution, and overall recovery of added vitamin was roughly 100%. Comparison with our standard laboratory procedures established a highly significant correlation coefficient with only a minor negative bias for folic acid and red cell folate (Fig. 2).

Antibiotic interference in the microbiological assay of folate was a problem noted in this evalua-

*Australian Wellcome Immunoassay Quality Control Program (Wellcome Diagnostics, Beckenham, England).

Table 6 Correlation of vitamin levels by Combostat II with ^{57}Co B_{12} plasma absorption and urinary excretion results or bone marrow examination results or both

Case no	Haemoglobin concentration (g/dl)	Mean cell volume (fl)	8h ^{57}Co B_{12} plasma absorption	24h ^{57}Co B_{12} urinary excretion	Bone marrow comments	Conclusion or final diagnosis	Vitamin B_{12} concentration (pmol/l)	Folate concentration (nmol/l)	Red cell folate concentration (nmol/l)
1	11.6	85	Ab†	Ab†	Hypocellular. Slight dyserythropoiesis. Stainable iron reduced	PA with iron deficiency	152	N	N
2	16.0	105	Ab†	Ab†	Megaloblastic change. Stainable iron reduced	PA with iron deficiency	56	N	N
3	9.6	106	Ab*	Ab*	Megaloblastic change. Stainable iron reduced	PA or MA with iron deficiency	54	N	N
4	6.9	94	NA	NA	Mild dyserythropoiesis. Nuclear/cytoplasmic asynchrony in erythroblasts	AML	N	4.5	N
5	8.9	75	NA	NA	Occ giant metamyelocytes. Prominent dyserythropoiesis. Stainable iron absent	Gastro intestinal blood loss PNH to be excluded	N	2.5	N
6	8.7	77	NA	NA	Slight dyserythropoiesis. Occ hypersegmented neutrophils	Myeloma	159	4.8	N
7	9.8	87	NA	NA	Megaloblastic change.	Megaloblastosis or drug effect	N	N	386
8	13.2	91	NA	NA	Moderate dyserythropoiesis. Occ hypersegmented neutrophils	?Early B_{12} or folate deficiency	N	4.3	386
9	9.8	102	NA	NA	Megaloblastic change.	? B_{12} /folate deficiency. ?Blood loss/haemolysis ?Primary dyserythropoietic anaemia	144	4.8	182
10	12.1	88	NA	NA	Micronormoblastic maturation. Stainable iron reduced. B_{12} /folate assays suggested	Reactive marrow with iron deficiency	N	N	306
11	4.4	115	Ab†	Ab†	Grossly megaloblastic	PA	22	N	159
12	7.8	58	NA	NA	Frankly megaloblastic. Stainable iron absent	?Combined deficiency	133	3.6	329
13	11.5	89	NA	NA	Moderate dyserythropoiesis	Myeloproliferative disease. Blastic transformation	N	N	272
14	5.9	103	Ab‡	Ab‡	Moderate dyserythropoiesis ?Azathioprine drug effect B_{12} /folate assays suggested	MA	96	N	N
15	10.8	123	N	Ab*	Megaloblastic (treated) change	PA or MA	144	4.8	182

N = normal.

Ab = abnormal.

NA = information not available.

PA = pernicious anaemia.

MA = malabsorption.

AML = acute myeloid leukaemia.

PNH = paroxysmal nocturnal haemoglobinuria.

*Results of repeat test with intrinsic factor not available.

†Corrected with intrinsic factor.

‡Did not correct with intrinsic factor.

tion. The unavailability of the clinical and drug histories of patients at the time of request is a common problem in this laboratory and would account for some of the falsely low results by non-commercial methods.

The clinical value of the Combostat II kit was in favourable agreement with the non-commercial methods and other more definitive clinical procedures. Detection rates between the methods in the deficient area were comparable, with few true discrepancies arising for each of the vitamin determinations. When a discrepancy did arise, the haematological data favoured the kit method. The correlation with bone marrow information and ^{57}Co B_{12} plasma absorption and urinary excretion results substantiated the kit's clinical reliability in the deficient region.

Definition of the laboratory reference range is of prime importance in the implementation of any clinical assay. Important considerations are the proper selection of individuals and that the sample number is sufficiently large so that valid statistical criteria may be satisfied. While non-parametric percentile methods for establishing the reference range make no assumptions as to the nature of the underlying sample population, they are not as powerful as parametric methods, when the sample population is indeed gaussian.⁸ Accordingly, data were transformed to meet the requirement of a normal gaussian distribution and the reference range was based on the transformed sample set (Table 3). The advantage of the latter is the greater confidence achieved at the end points of the range.⁸

Comparison of the manufacturer's ranges with those established above highlights the importance of determining a reference range within the laboratory. While the results of vitamin B_{12} were similar, those for folate and red cell folate differed substantially (Table 3). This type of disagreement does not necessarily reflect on the assay, but more so on the differences in populations based on geographic, dietary, and genetic factors. The contribution of an altered red cell folate haemolysate preparation on this disagreement is not known.

The cost of the dual radioisotope dilution assays is considerably more than that of non-commercial methods. As the radioisotope dilution assay method results in a saving in the labour requirement of our assay laboratory and allows mobilisation of resources into other areas of the laboratory, it offers a major advantage in manpower.

The use of a computer program to calculate all assay results is also beneficial. Rapid data reduction means that all results are available for reporting just 30 min after counting. Such instruments are also readily interfaced to counters effectively to automate the overall procedure.

On the basis of the evaluation contained in the report, we think that the Micromedic B_{12} /Folate Combostat II assay kit is technically simple to perform and yields results which are of similar clinical importance to the non-commercial methods. In addition, the results produced are reliable indicators of deficiency. We also believe that the introduction of a no boil solid phase technique, on which this method is based, has generally increased the standard of these assays, in particular vitamin B_{12} —a sentiment which is shared by others.⁴

References

- ¹ Lindenbaum J. Status of laboratory testing in the diagnosis of megaloblastic anaemia. *Blood* 1983;61:624-7.
- ² Schilling RF, Fairbanks VF, Miller R, Schmitt K, Smith MJ. "Improved" vitamin B_{12} assays: a report on two commercial kits. *Clin Chem* 1983;29:582-3.
- ³ El Shami AS, Durham AP. More on vitamin B_{12} results as measured with "Boil" and "No-boil" kits. *Clin Chem* 1983;29:2115-6.
- ⁴ Fish DI, Dawson DW. Comparison of methods used in commercial kits for the assay of serum vitamin B_{12} . *Clin Lab Haematol* 1983;5:271-7.
- ⁵ Olesen H. Vitamin B_{12} assays: Call for a definitive method. *Clin Chem* 1983;29:1442.
- ⁶ Waters AH, Mollin DL. Diagnosis of folate deficiency. *ACP Broadsheet* 79, 1973.
- ⁷ Tibbling G. A method for determination of vitamin B_{12} in serum by radioassay. *Clin Chim Acta* 1969;23:209-18.
- ⁸ Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. *Clin Chem* 1971;17:275-84.
- ⁹ Paltridge G, Rudzki Z, Ryall RG. Validity of transcobalamin II-based radioassay for the determination of serum vitamin B_{12} concentrations. *Ann Clin Biochem* 1980;17:287-92.
- ¹⁰ Cornbleet PJ, Cochman N. Incorrect least-squares regression coefficients in method-comparison studies. *Clin Chem* 1979;25:432-8.
- ¹¹ Dawson DW, Delamore IW, Fish DI, *et al.* An evaluation of commercial radioisotope methods for the determination of folate and vitamin B_{12} . *J Clin Pathol* 1980;33:234-42.

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