

Direct Assay for the Quantitative Assessment of Holo-Transcobalamin in Serum and Plasma

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INTRODUCTION

Accurate assays for determining vitamin B12 status are needed because of the high prevalence, particularly among the elderly, and serious complications of deficiency. Total plasma vitamin B12 concentration is the current standard clinical test for vitamin B12 deficiency. However, a proportion of individuals with vitamin B12 levels that would be considered deficient exhibit no clinical or biochemical evidence of deficiency conversely, neuropsychiatric and metabolic abnormalities can occur with plasma vitamin B12 concentrations well within the normal reference interval.

Holotranscobalamin (holoTC) is the biologically active portion of vitamin B12 available for cellular up-take. Less than 30% of the vitamin B12 in plasma circulates as holoTC; the remaining 70% is bound to haptocorrin which appears to be metabolically inert.

Recently a novel monoclonal antibody has been developed with 100-fold specificity for holoTC over TC which allows the development of simple, direct immunoassays for the quantitation of holoTC. Such assays also avoid the need for the various extraction steps employed by most vitamin B12 assays, so removing a possible cause of pre-analytical variability. Here we report the characteristics of a new holoTC enzyme immunoassay.

METHODS

Materials

HoloTC mAb was selected from a library of mouse hybridomas using primary screening for holoTC binding and secondary screening against apoTC binding. The high-affinity TC specific mAbs 3-9 and 3-11 were from State University of New York. Recombinant human holoTC and apoTC were from Cobento AS (Denmark).

The assay showed good precision as assessed in a 20 day CLSI study

Table 1

20 Day Precision on assay kit controls and QC samples

		Low Control	High Control	QC 1	QC 2	QC 3
Instrument 1	Mean (pmol/l)	22.8	47.9	27.5	38.5	59.1
	Total CV %	6.4	8.7	7.4	5.9	7.7
Instrument 2	Mean (pmol/l)	23.2	49.7	28.5	39.5	62.2
	Total CV %	6.6	7.9	7.9	7.1	9.3

The holoTC concentrations were determined in a clinically normal population (n=281) to determine the expected range of samples.

Table 2: 95% central reference limits of a normal population

	Lower limit pmol/L	Upper limit pmol/L
mean	19.2	120.0
95% confidence limits	19.0-19.4	117.5 - 122.1

The mean HoloTC concentration (derived from log-transformed data to normalize the population) was 47.9 pmol/L with a range from 8.9 to 123.4 pmol/L. The central 95% of the population defined by the expected range of 19.0 to 120.4 pmol/L. The lower limit of the central 95% reference interval is very similar to that found for the Axis Shield RIA HoloTC Assay (24pmol/l). Ref. *Marius Ulleland, et al; Clin. Chem., (2002); 48: 526 - 532.*

PA samples

The holoTC concentration in 5 confirmed pernicious anaemia patients was determined confirming extremely low holoTC concentrations

sample #	Mean HoloTC (pmol/l)	Total B ₁₂ (pmol/l)
1	2.3	62
2	3.4	92
3	4.3	62
4	2.8	87
5	3.8	49

Table 3 100% of pernicious anaemia samples (n=5) were classed as very low deficient by the AxSYM assay.

Analysis of routine samples

720 consecutive samples submitted for B₁₂ analysis (kindly provided by Furst Lab, Oslo) were also assayed for holoTC concentration. The B₁₂ values were stratified as below B12 cut-off (170pM), 170-300pM as samples in the potential indeterminate zone for B₁₂ and >300pM B₁₂ to represent B₁₂ replete.

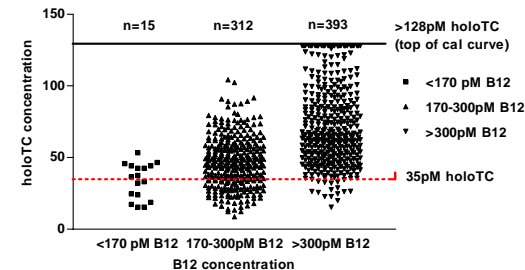


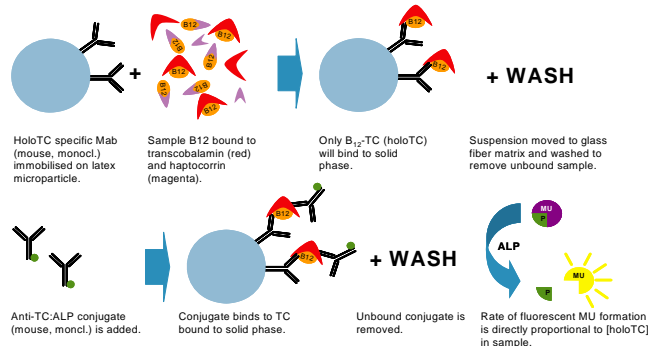
Fig 2. Approximately 43% of all the samples analysed fall into the indeterminate zone for total B₁₂ concentration; 25% of the samples in this indeterminate zone exhibit abnormal holoTC levels.

CONCLUSION

With the identification of a monoclonal antibody highly specific for holoTC, it becomes possible to construct direct assays for holoTC adaptable for use on the larger clinical instruments. Using the Abbott AxSYM system, a precise and rapid has been developed.

AxSYM® HoloTC reaction schematics

2-Step sandwich MEIA



The assay was compared to the predicate device: the Axis-Shield RIA

Fig. 1

Passing-Bablok Analysis n=204 r=0.9

