Short Communication

Vitamin B12 absorption judged by measurement of holotranscobalamin, active vitamin B12: evaluation of a commercially available EIA kit

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Abstract

Background: Active vitamin B12 absorption is followed by an increase in holotranscobalamin (holoTC) upon loading with a high physiological dose of the vitamin (the CobaSorb test). This study evaluates the use of a newly launched EIA kit for measurement of holoTC (active B12) in relation to the CobaSorb test.

Methods: Intra-assay imprecision and linearity of the EIA kit was examined, employing serum pools of increasing holoTC concentrations. For the CobaSorb test, holoTC was measured before and after loading with 3-times 9 µg of vitamin B12 employing both the in-house ELISA and the EIA kit (n=25).

Results: The EIA kit showed an intra-assay CV between 2.2% and 5.8% for holoTC values ranging from 21 to 80 pmol/L. Employing diluted serum samples resulted in spurious high values of holoTC. The EIA kit performed well in relation to the CobaSorb test and classified the patients studied as capable of absorbing vitamin B12 (n=10) or not (n=15), as did the in-house ELISA.

Conclusions: The Active B12 (holoTC) EIA kit proved suitable for use with the CobaSorb test, but not for analysis of diluted serum samples.

Keywords: active B12; B12 absorption; holotranscobalamin; method evaluation.

Recently, we launched a new vitamin B12 (B12) absorption test named CobaSorb (1–3). In the final design of the test we measured the active part of plasma B12, holotranscobalamin (holoTC), before and after 2 days loading with an oral dose of 3-times 9 µg of B12. We judged the patient able to absorb the

vitamin if holoTC increased more than 10 pmol/L and more than 22% (2). The test has proven clinically useful in order to identify individuals not in need of treatment with pharmacological doses of B12 (4). A major drawback has been the lack of a suitable commercially available method for measurement of holoTC (active B12) applicable both in routine and in smaller specialized laboratories.

Now a manual active B12 EIA version of the holoTC method available on the AxSYM platform from Abbott (5) has been launched (Axis-Shield Diagnostics, Dundee, Scotland, UK). The advantage of the test is that it directly measures the B12 saturated part of transcobalamin (TC), holoTC or active B12 (6). In comparison, our in-house ELISA measures the amount of TC that remains after removal of unsaturated protein (7, 8). Here, we validate the new EIA kit and show that this assay may be useful for exploring B12 absorption as judged by CobaSorb.

Throughout the study we used leftover serum samples from the routine laboratory or from previous studies of vitamin B12 absorption (1). Based on previous studies (8) and our in-house control sample data (data not shown); holoTC is stable in storage at –20°C for years. The investigated samples were kept frozen at –20°C for up to 6 years before analysis. Because none of the samples could be traced to patient data there was no request for approval by the scientific Ethical Committee. We used the Axis-Shield active B12 EIA kit (9) and diluted samples 1:1 in sample diluents according to the kit insert. In addition, we used our in-house ELISA (7, 10). The two samples obtained as part of the CobaSorb test are always run on the same day and, because of that, our main interest was to explore the intra-assay variation of the EIA kit. We examined the intra-assay imprecision and linearity of the EIA kit by mixing serum pools with high and low levels of holoTC. All pools were run 10-times on each of 2 days.

The EIA assay showed an excellent linearity and an intra-assay imprecision of 2.2%–5.8% (Figures 1A and B). Dilution of the 40 pmol/L pool with assay buffer resulted in spurious high values (Figure 1A). To further explore the feasibility of diluting serum samples, we diluted six sera with assay buffer and compared expected values to those obtained. All six samples failed to dilute in a linear manner and all gave higher results than expected upon dilution (Figure 1C).

We examined samples from patients tested for B12 absorption employing the CobaSorb test (n=25), including also samples from eight patients with an inherited inability to

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Figure 1 Evaluation of the EIA kit for measurement of holoTC (active B12) in relation to the CobasorB test.

(A) Serum pools with a high ($a=80$ pmol/L) and with a low ($e=40$ pmol/L) level of holoTC as determined by the EIA holoTC method were mixed to get the expected holoTC concentrations of 40 (e), 50 (d), 60 (c) and 70 (b) pmol/L. In addition, a sample with an expected holoTC level of 20 pmol/L was prepared by 2-fold dilution of the (e) pool with kit assay buffer [indicated by a circle (f) on (A)]. Levels of holoTC were measured 20 times over 2 days by the EIA kit and results obtained were plotted as a mean with intra-assay SD against the expected values. The intra-assay imprecision (CV%) calculated for each of the serum pools is indicated. The linear regression line [$y=0.99 (\pm 0.02)+0.67 (\pm 1.0)$] for the serum matrix samples show a slope and intercept not deviating from 1 and 0. (B) Serum pools with a high level (80 pmol/L) and with a low level (21 pmol/L) of holoTC as determined by the EIA holoTC method were mixed to give the expected values of 51 (g), 36 (h), 28 (j) and 21 (k) pmol/L and evaluated as indicated for (A). The linear regression line ($y=1.22 (\pm 0.03) - 6.2 (\pm 1.4)$) shows a slope and intercept deviating from 1 and 0. (C) Six serum samples were diluted with assay buffer 2-, 4- and 8-fold. The results obtained were plotted against the expected. (D) Results from the CobasorB test. Blood samples removed from 17 patients prior to and after 2 days intake of 3-times 9 μg of B12. In addition, samples from eight patients with inherited inability to absorb vitamin B12 (1) were analysed. All samples were analysed both by the EIA kit and the in-house ELISA. Results from the EIA kit are shown. We used our in-house ELISA to classify the patients as able to absorb B12 (holoTC increased more than 10 pmol/L and more than 22%), $n=10$ (full drawn line), or not, $n=15$ (dashed or dotted lines). The latter group included the eight patients (dotted lines) with inherited inability to absorb vitamin B12. All patients were classified correctly by the EIA holoTC assay according to the same criteria as used for classifying by the ELISA assay.

absorb the vitamin (1). All patients had baseline holoTC below 65 pmol/L (3). The patients were classified as absorbers if holoTC increased more than 10 pmol/L and more than 22% (2). The in-house ELISA and the EIA resulted in an identical classification of the patients, 10 were classified as absorbers and 15 were classified as unable to absorb the vitamin (Figure 1D).

We evaluated the use of a commercially available assay for measurement of holoTC, active B12, as part of the vitamin B12 absorption test CobasorB. Since the two samples obtained as part of this test are analyzed together, our focus was an evaluation of the intra-assay imprecision. Because of

that our evaluation cannot be used to judge the usefulness of the test for diagnostic purposes. Our evaluation shows the assay to have a sufficient intra-assay imprecision ranging from 2.2% to 5.8% for holoTC values between 20 and 80 pmol/L. The assay can only be employed on serum samples that have been diluted 1:1, as recommended by the manufacturer, since additional dilutions result in spurious high values for holoTC. Despite these limitations we found the new EIA method to perform well in relation to the CobasorB test.

We conclude that the EIA kit for measurement of holoTC (active B12) may prove suitable for use with the CobasorB test, but not for analysis of diluted serum samples.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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