



# Multicentre evaluation of the Roche Elecsys<sup>®</sup> Active B<sub>12</sub> (holotranscobalamin) electro-chemiluminescence immunoassay

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## Abstract

**Background:** Vitamin B<sub>12</sub> deficiency is a common disorder. In circulation, vitamin B<sub>12</sub> is bound to transcobalamin (holotranscobalamin), which is considered the active form of cobalamin. The objective of this study was to evaluate the analytical performance of the Roche Elecsys Active B<sub>12</sub> immunoassay.

**Methods:** Limit of quantification and linearity were assessed according to CLSI EP17-A2 and EP-6A guidelines. Precision and bias of Roche Active B<sub>12</sub> test against Architect ci8200 (Abbott) were performed according to CLSI EP-5 A3 guideline at three European laboratories. Precision requirements were set at <4% for within-run precision and <15% for inter-mediate precision. Requirements for method comparison were set at a slope of 1.00 ± 20%. In addition, 95% reference interval was conducted in European adults according to CLSI-EP28 guideline.

**Results:** The assay was shown to be linear and precise and met the requirements. Lot-to-lot variation and lab-to-lab variation both were ≤4%. Method comparison with Abbott Architect showed a significant bias of 9 pmol/L, which increases to up to 23 pmol/L around the current medical decision point. The 95% reference interval of the Roche Elecsys Active B<sub>12</sub> test was determined at 37–188 pmol/L.

**Conclusion:** Although Roche Elecsys Active B<sub>12</sub> immunoassay met the requirements, this test has a positive bias of 9 pmol/L, which led to an increase in lower limit of reference interval compared with Abbott test. This test first needs to be clinically validated before it can be applied as a first-line screening test in the diagnosis of vitamin B<sub>12</sub> deficiency.

## Keywords

Method evaluation, reference interval, transcobalamin, vitamin B<sub>12</sub>, vitamin B<sub>12</sub> deficiency

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## Introduction

Vitamin B<sub>12</sub> deficiency is common and occurs particularly among the elderly. The diagnosis of vitamin B<sub>12</sub> deficiency is hampered by the availability of good biomarkers. Clinical vitamin B<sub>12</sub> deficiency with classical haematological and neurological symptoms also known as pernicious anaemia is relatively uncommon and is estimated to affect 2–3% of individuals of

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65 years and older.<sup>1-3</sup> More often, subclinical vitamin B<sub>12</sub> deficiency occurs which, depending on the chosen cut-off value of vitamin B<sub>12</sub>, ranges from 3% to 26% in the general population.<sup>4</sup>

Vitamin B<sub>12</sub> is mainly derived from animal sources, and after ingestion, it is bound to haptocorrin in the stomach. In the duodenum, vitamin B<sub>12</sub> is released from haptocorrin and binds to intrinsic factor after which it is taken up in the enterocytes of the distal ileum. In circulation, about 20% of vitamin B<sub>12</sub> is bound to transcobalamin (holotranscobalamin, holoTC), which is the active form of vitamin B<sub>12</sub> available to cells, while the remaining 80% is bound to haptocorrin.

The diagnosis of vitamin B<sub>12</sub> deficiency is hampered by the availability of biomarkers with good diagnostic performance. Most frequently, total vitamin B<sub>12</sub> is measured in serum or plasma as first-line test to estimate an individual's vitamin B<sub>12</sub> status. Several years ago, the active B<sub>12</sub> assay became available, which enabled the measurement of holoTC on the Abbott routine platform. As this holoTC assay is not hampered by fluctuations in haptocorrin, it was expected to result in better diagnostic performance. Several studies have assessed the diagnostic performance of holoTC (or active B<sub>12</sub>) and have shown a marginally better performance of the active B<sub>12</sub> test compared with total vitamin B<sub>12</sub>.<sup>5-7</sup> In addition, homocysteine and methylmalonic acid (MMA) are frequently used as functional markers of vitamin B<sub>12</sub>.<sup>3,8,9</sup> Most laboratories use total vitamin B<sub>12</sub> or active B<sub>12</sub> as first-line test, which is followed by MMA or homocysteine as second-line test<sup>9</sup>. The medical decision point is based at either an active B<sub>12</sub> <20 pmol/L or in case active B<sub>12</sub> is between 20 and 35 pmol/L by a follow-up test like MMA or homocysteine. For the diagnosis of vitamin B<sub>12</sub> deficiency, it is of utmost importance to have access to biomarkers for vitamin B<sub>12</sub> deficiency such as total vitamin B<sub>12</sub> and active B<sub>12</sub>. At this moment, active B<sub>12</sub> is available at Abbott Architect and was recently introduced by Siemens ADVIA centaur and Roche Diagnostics, which facilitates the availability of active B<sub>12</sub> in clinical chemistry laboratories.

In this study, we performed a multicentre analytical evaluation of the Roche Elecsys® Active B<sub>12</sub> immunoassay and assessed linearity, precision and bias by method comparison between **cobas e 411** (Roche Diagnostics) and Architect ci8200 (Abbott) and determined the 95% reference interval in European individuals.

## Material and methods

This study was executed between October 2016 and May 2017. Analytical performance of the Active B<sub>12</sub> assay was evaluated on a **cobas e 411** analyzer

(Roche Diagnostics GmbH, Penzberg, Germany). Precision and method comparison were evaluated in three laboratories; Labor Limbach in Heidelberg, Germany, Erasmus MC, Rotterdam, The Netherlands and Synlab, Munich, Germany. Precision was evaluated in all three laboratories, and method comparison was evaluated in two laboratories that used WHO 03/178 standardized Abbott reagents, i.e. Heidelberg and Rotterdam.

### Analytical performance

Analytical performance of the Roche Active B<sub>12</sub> immunoassay was assessed at **cobas e 411** using reagent lot 245198 99 (Roche Diagnostics GmbH, Penzberg, Germany).

*Limit of Blanc, limit of detection, limit of quantitation.* Limit of Blanc (LoB) and limit of detection (LoD) were determined by measuring 10 times an analyte-free sample in six runs. LoB and LoD were calculated according to CLSI EP17-A2. Limit of quantitation (LoQ) was calculated according to CLSI EP17-A2; eight samples with low concentrations (between 3.2 and 8.7 pmol/L, respectively) were measured in five-fold on five days, and the coefficient of variation (CV%) was calculated per sample. LoQ is defined at the lowest concentration at which CV <10%.

*Linearity.* Linearity of the Active B<sub>12</sub> assay was assessed by diluting three high analyte serum samples with diluent. Fifteen concentrations from above the upper limit throughout the measuring range and below the lower end of the specified measuring range were prepared (measuring range; between 3 and 150 pmol/L). The dilutions were measured in three-fold within a single run using one lot. Evaluation was performed according to CLSI EP6A guideline using a validated software tool provided by Roche Diagnostics Penzberg Biometry department. All the measured data of each dilution series were analysed in a respective regression analysis. Data were analysed using linear, quadratic and cubic order least square regression analysis according to CLSI guidelines. Specifications were predefined at ≤0.8 pmol/L at a concentration below 8 pmol/L and <10% for concentrations between 8 and 150 pmol/L.

*Interference.* Interference of the Active B<sub>12</sub> assay for bilirubin, haemoglobin, intralipids and biotin was assessed by diluting a sample without interfering substance with a sample with interfering substance. For bilirubin interference, this was assessed at three concentrations 32.9, 74.2 and 145 pmol/L of active B<sub>12</sub> using a serum sample with 66 mg/dL (1129 μmol/L) bilirubin. Haemoglobin interference was assessed at 29.7, 71.5

and 137 pmol/L of active B<sub>12</sub> using a serum sample with 1000 mg/dL (0.621 mmol/L) haemoglobin. Intralipid interference was assessed at 27.3, 65.6 and 125 pmol/L using a serum sample with 2000 mg/dL intralipids. Biotin interference was assessed at 34.1, 70.4 and 120 pmol/L active B<sub>12</sub> using a serum sample with 100 ng/mL biotin. Recovery was calculated, and for analyte concentrations of 3–8 pmol/L, the maximum allowed deviation was specified at 0.8 pmol/L; for concentrations between >8–100 pmol/L, the deviation was set at ≤10%, and for concentrations >100 pmol/L, the deviation is set at ≤15%.

**Sample stability.** Sample stability at –20°C was determined by calculating the recovery of four human serum samples with different active B<sub>12</sub> concentrations (i.e. 13, 44, 76 and 141 pmol/L) in triplicate for six months at –20 ± 5°C). Specification for recovery was set at ±10% for analyte concentrations >8 pmol/L.

### *Multicentre validation*

**Precision.** Precision of the Elecsys® Active B<sub>12</sub> immunoassay was assessed at each laboratory on a **cobas e 411** analyzer (Roche Diagnostics GmbH, Penzberg, Germany) by measuring the same set of samples; two levels of controls (PeciControl Active B<sub>12</sub>, provided by Roche Diagnostics GmbH, Penzberg, Germany) and five concentrations of pooled human serum samples (provided by Roche Diagnostics GmbH, Penzberg, Germany) with two reagent lots and one Calset calibrator set consisting of two levels of calibrators at the **cobas e 411** analyzers according to CLSI-EP5 A3 protocol. At each laboratory, five replicates of each sample (PeciControl and human serum pools) were measured for five days in one run per day for each reagent lot (1980 Lot and 1990 lot, provided by Roche Diagnostics GmbH, Penzberg, Germany). All samples used for precision were shipped on dry ice and were stored at –20°C before testing. Intermediate precision of Abbott Architect Active B<sub>12</sub> was reported <6% (Abbott Architect Active-B<sub>12</sub>, package insert October 2015). Within-precision and intermediate precision were calculated for each laboratory using Excel (version 2.30). Lot-to-lot variation was calculated by calculating the mean of each lot per laboratory after which the mean, standard deviation and coefficient of variation of the two lots were calculated. Lab-to-lab variation was calculated by calculating individual means of a single lot (1980) for each laboratory after which the mean, standard deviation and coefficient of variation were calculated.

**Bias.** At each laboratory, a minimal amount of 120 anonymized left-over human serum patient samples, in which active B<sub>12</sub> was routinely measured by the

Architect Active B<sub>12</sub> assay (Abbott, Architect, Chicago, USA), were collected and stored at –20°C until measurement. Samples were collected at each laboratory according to predefined percentages covering the measuring range: ≥10% <25 pmol/L; ≥30% with active B<sub>12</sub> between 25 and 50 pmol/L; ≥20% with active B<sub>12</sub> between 50 and 100 pmol/L and ≥10% of active B<sub>12</sub> >100 pmol/L. One reagents lot of Roche Elecsys immunoassay (MP lot, Roche Diagnostics, standardized against WHO 03/178), one level of Roche CalSet Active B<sub>12</sub> calibrators consisting of two levels of calibrators and two levels of Roche PeciControls were used for method comparison). Two lots of Abbott active B<sub>12</sub> reagents (both traceable against the WHO 03/178 [lot 10556UP00] [Erasmus MC] and lot 10557UP00 [Heidelberg]), four levels of controls (two commercial controls and two in-house serum pools) and two calibrator sets consisting each of six levels of calibrators were used in this study. Samples were thawed at room temperature and were homogenized gently by inverting the tubes and were centrifuged at 4000 g for approximately 4 min. The supernatant then was transferred into a new qualified tube immediately and was used for testing. Each sample was measured at the same day on both **cobas e 411** as Architect ci8200 analyzer at ≥1 run per day and ≥1 days. Weighted Deming regression was applied by method comparison function in Analyse-it for Excel (version 2.30). Bland-Altman bias plots were obtained by Analyse-it for Excel plotting the absolute difference between **cobas e 411** (Roche Diagnostics) and Architect ci8200 (Architect) in pmol/L against mean active B<sub>12</sub> concentrations in pmol/L. Normality of residuals was assessed by visual inspection of the histogram.

### *Reference interval*

Serum samples were collected from apparently healthy adults at one site operated by the German Blood Transfusion Service in Bavaria, Germany. Two hundred and fifty-two samples with approximately 50%–50% male–female distribution and with age from 20 to 79 had been selected. Homocysteine concentrations were determined in all samples using **cobas** Homocysteine assay (Roche Diagnostics). Only samples with homocysteine concentration <15 μmol/L were selected for Active B<sub>12</sub> measurements. In total, 214 of 252 samples qualified, and these were randomized and sent to all three laboratories. Two reagent lots have been used, with MP Lot at Erasmus MC and Heidelberg and PoQ Lot at Munich. For all samples with measurement results above measuring range, a 1:2 dilution was made and the samples were measured again. As only 1:2 dilution is allowed by the package insert, all samples that are still above measuring limit of

150 pmol/L after 1:2 dilution are considered '>300 pmol/L' in the analysis. In total, one sample had an active B<sub>12</sub> concentration >300 pmol/L. 95% reference ranges were calculated using non-parametric methods in accordance with CLSI EP28-A3.<sup>a</sup>

## Results

### Analytical performance

In this experiment, the LoB was calculated to be 0.66 pmol/L and LoD was 0.78 pmol/L. The LoQ of this experiment demonstrated a CV of 2.9% at the lowest tested active B<sub>12</sub> concentration of 3.2 pmol/L. From these results, the lower limits of measurements for the Elecsys Active B<sub>12</sub> assay were decided to be set at: LoB = 2.0 pmol/L, LoD = 3.0 pmol/L and LoQ = 5.0 pmol/L. Samples were shown to be linear between 3.0 and 150 pmol/L active B<sub>12</sub> with an absolute deviation of ≤0.8 pmol/L in the lower range (3–8 pmol/L) and a relative deviation of ≤10% in the range between 8 and 150 pmol/L. Interference for bilirubin was not detected up to a concentration of 66 mg/dL (1129 μmol/L). For haemoglobin, intralipids and biotin interference could not be detected up to concentrations of 1000 mg/dL (0.621 mmol/L) haemoglobin, 2000 mg/dL intralipids and 40 ng/mL biotin (164 nmol/L). Samples were stable at –20°C for six months.

### Precision

Two data points from the human serum pool (HSP05 were excluded from the analysis (Munich site, HSP05, 1 from MP Lot and 1 from PoQ lot), as they were above the measuring range. All other data points were used for precision analysis. Within-run precision of Roche Elecsys® Active B<sub>12</sub> immunoassay was <4% at each centre, and intermediate precision was <6% at each centre (Table 1). Lot-to-lot variation was <4% and lab-to-lab variation was <3.1%.

### Bias

One hundred and forty-seven samples were used for method comparison at the Heidelberg site and 142 different samples were measured at the Erasmus MC site. Method comparison of cobas e 411 compared with Architect ci8200 resulted at the Heidelberg site in a constant bias of 11 pmol/L and a proportional bias of 10% (0.90 [95%CI 0.83–0.98] x + 11.28 [95%CI 8.28–14.27], R = 0.94, P < 0.0001, n = 147). At the Erasmus MC site, this resulted in a significant constant bias of 8 pmol/L and no significant proportional bias (0.99 [95%CI 0.88–1.09] x + 7.52 [95% CI 3.20–11.84], R = 0.93, P < 0.0001, n = 142). Combined results from Heidelberg and Rotterdam resulted in a small

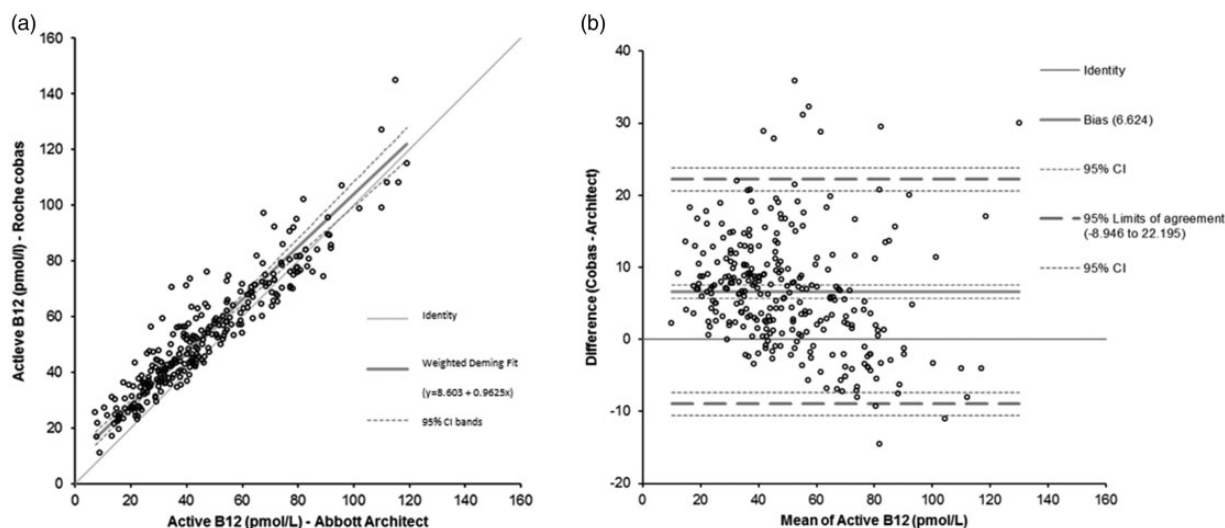
**Table 1.** Within precision and intermediate precision (IM) of Roche Elecsys® Active B<sub>12</sub> assay at three sites.

	Rotterdam			Heidelberg			Munich			Lab-to-lab	
	Mean <sup>a</sup> pmol/L	Within	IM	Mean	Within	IM	Mean	Within	IM	Lot-to-lot <sup>b</sup> CV (%)	CV (%)
		CV (%)	CV (%)		CV (%)	CV (%)		CV (%)	CV (%)		
HSP1	26.7	1.4	1.9	28.1	2.6	2.8	28.2	2.2	4.8	1.8	3.1
HSP2	46.9	1.3	1.8	48.5	1.5	2.7	49.6	3.6	5.1	1.2	2.8
HSP3	71.6	1.4	1.9	74.5	1.2	2.3	75.2	2.3	5.0	1.4	2.6
HSP4	106.1	1.7	2.3	114.6	2.2	3.1	112.2	3.0	5.8	1.0	4.0
HSP5	131.9	0.9	1.5	140.6	1.7	2.8	139.2	2.7	5.2	1.3	3.4
CON1	34.2	1.8	2.5	35.9	2.0	3.4	35.5	2.6	5.6	0.9	2.6
CON2	55.2	1.3	2.0	56.0	0.8	2.7	57.5	2.5	5.2	1.5	2.1

<sup>a</sup>Mean of five samples measured for five days with lot 1980.

<sup>b</sup>Lot-to-lot variation of lot 1980 versus lot 1990.

HSP: human serum pool; CON: PreciControl Active B<sub>12</sub>; IM: intermediate precision.



**Figure 1.** Bias plot of active B<sub>12</sub> assay at Roche **cobas e 411** compared with Abbott Architect. (a) Weighted Deming regression of active B<sub>12</sub> at Roche **cobas e 411** plotted against Abbott Architect ci8200. (b) Bland-Altman bias plot showing the absolute difference between **cobas e 411** and Architect ci8200 plotted against mean of active B<sub>12</sub> in pmol/L.

significant constant bias of 9 pmol/L and no significant proportional bias compared with Abbott Architect ( $y = 0.96$  [95%CI 0.88–1.02] $x + 8.6$  [95% CI 5.8–11.4],  $R = 0.94$ ,  $P < 0.001$ ,  $n = 289$ , Figure 1(a)). The difference plot of combined results of Rotterdam and Heidelberg shows a significant constant bias of 6.6 pmol/L (95% CI 5.7 pmol/L till 7.5 pmol/L) compared with Abbott Architect (Figure 1(b)) with an agreement range of  $-8.9$ – $22.2$  pmol/L. This bias shows a negative trend proportional to the magnitude of measurement showing a higher bias when the active B<sub>12</sub> concentration becomes lower.

### Reference interval of active B<sub>12</sub>

Median active B<sub>12</sub> concentration in serum of 214 samples was 77 pmol/L. The 95% reference interval of Roche Active B<sub>12</sub> assay was calculated at 37–188 pmol/L.

### Discussion

Active B<sub>12</sub> is commonly used as biomarker in the diagnosis of vitamin B<sub>12</sub> deficiency. In this study, we evaluated the new Roche® Elecsys Active B<sub>12</sub> assay at the **cobas e 411** instrument at three European sites and showed that the assay is linear and precise but shows a bias around the current medical decision range (20–35 pmol/L) based on Abbott Architect.

The Roche® Elecsys Active B<sub>12</sub> assay was shown to be precise with an intermediate precision lower than 6%, which is comparable to Abbott Architect intermediate precision of <6% (Abbott Architect Active-B<sub>12</sub>, package insert October 2015). Lab-to-lab variation and

lot-to-lot variation both were small, enabling harmonization of test results and facilitating the use of general cut-off values in clinical algorithms. Method comparison showed a small constant bias of 9 pmol/L. Visual inspection of the bias plot shows that the observed bias is not constant over the measuring range. Roche Active B<sub>12</sub> results within the lower range have a larger bias, which is expected to result in a higher active B<sub>12</sub> test result (up to 22 pmol/L) around the current clinical decision point of 20–35 pmol/L.

Current diagnosis of vitamin B<sub>12</sub> using active B<sub>12</sub> is in most laboratories a two-step process, in which active B<sub>12</sub> is applied as first-line test. If active B<sub>12</sub> is <21 pmol/L, then the diagnosis of vitamin B<sub>12</sub> deficiency is confirmed if clinical signs are present. However, if the test result is between 21 and 35 pmol/L, a follow-up test is performed by measuring MMA to confirm diagnosis because specificity of the active B<sub>12</sub> test is too low within this range. Patients with active B<sub>12</sub> > 35 pmol/L are unlikely to be diagnosed with vitamin B<sub>12</sub> deficiency. However, these cut-off values are mostly based on the Abbott Active B<sub>12</sub> test. In this study, however, we have shown that the reference interval of Roche Active B<sub>12</sub> is considerably higher (37–188 pmol/L) making the current cut-off values not suitable. Novel cut-off values of Roche Active B<sub>12</sub> should be established by calculating sensitivity and specificity of active B<sub>12</sub> concentrations against MMA concentrations before this test can be applied as first-line screening test in the diagnosis of vitamin B<sub>12</sub> deficiency.

In future, clinical algorithms would benefit from standardization against an active B<sub>12</sub> reference method using commutable standards in the assay.

This multicentre evaluation of the Roche Elecsys Active B<sub>12</sub> assay demonstrates excellent precision. A bias compared with Abbott Architect Active B<sub>12</sub> assay was observed, which needs to be accounted by establishment of laboratory-specific reference intervals. Before this assay can be implemented on Roche routine platforms, a clinical validation of the Roche active B<sub>12</sub> test is needed to establish reliable cut-off values to define clinical- and subclinical vitamin B<sub>12</sub> deficiency.

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#### Declaration of conflicting interests

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#### Ethical approval

The medical ethical committee of the Erasmus MC declared that this project is not subject to rules on medical research involving human subjects as anonymized left-over material is used (ref MEC-2016-599). In addition, approval has been received for collection of patient samples for reference interval study (Ethik Kommission der Bayerischen Landesärztekammer in Munich, Germany).

#### Guarantor

SGH.

#### Contributorship

SGH supervised the study at the Erasmus MC site, conducted data analysis, gained ethical approval and drafted the article. PB supervised the study at the

Munich site. PF supervised the study at the Heidelberg site. SL developed the Roche active B<sub>12</sub> assay and supervised the analytical measurements at Roche Diagnostics and was involved in data analysis and YS wrote the study protocol and supervised the study at Roche Diagnostics. YBdR contributed to data interpretation and writing of the article. All authors corrected and approved the final version of the article.

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#### Note

a. Values in this article may slightly differ from the Roche® Active B<sub>12</sub> immunoassay package insert due to the use of different software packages.

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