

Elecsys Vitamin B12 II

REF		SYSTEM
07212771 190	100	MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

English

System information

For **cobas e 411** analyzer: test number 1410
 For MODULAR ANALYTICS E170, **cobas e 601** and **cobas e 602** analyzers: Application Code Number 224

Intended use

Binding assay for the in vitro quantitative determination of vitamin B12 in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Vitamin B12, also referred to as cobalamin, is a complex organometallic compound in which a cobalt atom is situated within a corrin ring. It is a water-soluble vitamin which is synthesized by microorganisms. It cannot be synthesized in the human body and is seldom found in products of plant origin. Main sources of vitamin B12 are meat, fish, eggs and dairy products.¹ The uptake in the gastrointestinal tract depends on intrinsic factor, which is synthesized by the gastric parietal cells, and on the "cobam receptor" in the distal ileum. The most frequent cause of severe vitamin B12 deficiency is a lack of intrinsic factor due to autoimmune atrophic gastritis. The disease is historically called "pernicious anemia", even though many patients present with mainly neurologic manifestations. Examples of other causes for vitamin B12 deficiency are malabsorption due to gastrectomy, inflammatory bowel disease or dietary deficiency, e.g. in strict vegetarians (vegans).²

Vitamin B12 is the cofactor for two enzymes, methionine synthase and methylmalonyl CoA mutase.^{2,3} Methionine synthase, located in the cytoplasm, requires vitamin B12 in the form of methylcobalamin and catalyzes the conversion of homocysteine to methionine, an essential amino acid. During this step a methyl group is transferred from methyltetrahydrofolate to the amino acid.³ This enzyme links the methylation pathway through synthesis of the methyl donor S-adenosyl methionine and the pathway in which purine and pyrimidine are synthesized via generation of tetrahydrofolate.³ In the form of 5'-deoxyadenosylcobalamin, vitamin B12 is also required for the mitochondrial enzyme methylmalonyl CoA mutase, which converts methylmalonyl CoA to succinyl CoA. This is a step in the oxidation of odd-chain fatty acids and catabolism of ketogenic amino acids.³ Thus, vitamin B12 is important for DNA synthesis, regenerating methionine for protein synthesis and methylation, as well as for the development and initial myelination of the central nervous system (CNS) and for the maintenance of normal CNS function.^{2,3}

Vitamin B12 deficiencies are common in wealthier countries principally among the elderly and are most prevalent in poorer populations. In general the prevalence increases with age.^{4,5}

Vitamin B12 deficiency impacts red blood cell synthesis, resulting in megaloblastic anemia due to abnormal DNA synthesis.³ In addition it impairs neurological function, in particular demyelination of nerves in part due to abnormal methylation, leading to peripheral neuropathy, dementia, poor cognitive performance, and depression.³ Other effects of vitamin B12 deficiency or depletion are increased risk of neural tube defects, osteoporosis, cerebrovascular and cardiovascular diseases.³ Early diagnosis is essential, because of the latent nature of this disorder and the risk of permanent neurological damage.^{3,5}

Generally, the primary test performed to confirm the diagnosis of vitamin B12 deficiency is measurement of serum vitamin B12 level.² Recent publications suggest that in addition the following biomarkers should be measured to improve the specificity of diagnosis: folate, methylmalonic acid (MMA), homocysteine and holotranscobalamin.^{2,5,6,7}

The Elecsys Vitamin B12 II assay employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample competes with the added vitamin B12 labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex^{a)}.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (15 µL) with the vitamin B12 pretreatment 1 and pretreatment 2, bound vitamin B12 is released.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor vitamin B12 biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents - working solutions

The reagent rackpack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as B12 II.

PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL:

Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.

PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL:

Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Intrinsic factor-Ru(bpy)₃²⁺ (gray cap), 1 bottle, 10 mL:

Ruthenium labeled recombinant porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.

R2 Vitamin B12-biotin (black cap), 1 bottle, 8.5 mL:

Biotinylated vitamin B12 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



Danger

H290

May be corrosive to metals.

Elecsys Vitamin B12 II

H314 Causes severe skin burns and eye damage.

H412 Harmful to aquatic life with long lasting effects.

Prevention:

P273 Avoid release to the environment.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P301 + P330 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
+ P331

P303 + P361 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
+ P353

P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
+ P310 Immediately call a POISON CENTER/ doctor.

P305 + P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
+ P338 Continue rinsing. Immediately call a POISON CENTER/ doctor.
+ P310

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{8,9}

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	84 days (12 weeks)
on the analyzers	35 days (5 weeks) onboard or 60 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 10 x 8 hours

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Na-heparin, Li-heparin, K₂-EDTA and K₃-EDTA plasma. Li-heparin plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within $\pm 2 \times$ Limit of Blank + coefficient of correlation ≥ 0.95 .

Stable for 2 hours at 15-25 °C, 48 hours at 2-8 °C, 56 days at -20 °C (± 5 °C). Freeze once only.

Stability of serum obtained with separating tubes: 24 hours at 2-8 °C (note the data provided by the tube manufacturer).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Avoid hemolysis.

Do not use samples and controls stabilized with azide.

Vitamin B12 determinations should be performed on serum or plasma samples from fasting patients.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- [REF 07212780190](#), Vitamin B12 II CalSet, for 4 x 1.0 mL
 - [REF 05618860190](#), PreciControl Varia, for 4 x 3.0 mL
 - [REF 11732277122](#), Diluent Universal, 2 x 16 mL sample diluent or
[REF 03183971122](#), Diluent Universal, 2 x 36 mL sample diluent
 - General laboratory equipment
 - MODULAR ANALYTICS E170 or **cobas e** analyzer
- Accessories for **cobas e** 411 analyzer:
- [REF 11662988122](#), ProCell, 6 x 380 mL system buffer
 - [REF 11662970122](#), CleanCell, 6 x 380 mL measuring cell cleaning solution
 - [REF 11930346122](#), Elecsys SysWash, 1 x 500 mL washwater additive
 - [REF 11933159001](#), Adapter for SysClean
 - [REF 11706802001](#), AssayCup, 60 x 60 reaction cups
 - [REF 11706799001](#), AssayTip, 30 x 120 pipette tips
 - [REF 11800507001](#), Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- [REF 04880340190](#), ProCell M, 2 x 2 L system buffer
- [REF 04880293190](#), CleanCell M, 2 x 2 L measuring cell cleaning solution
- [REF 03023141001](#), PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- [REF 03005712190](#), ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- [REF 03004899190](#), PreClean M, 5 x 600 mL detection cleaning solution
- [REF 12102137001](#), AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- [REF 03023150001](#), WasteLiner, waste bags
- [REF 03027651001](#), SysClean Adapter M

Accessories for all analyzers:

- [REF 11298500316](#), ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

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The values shown below were performed on samples from an apparently healthy population, using the Elecsys Vitamin B12 II assay. The calculation is based on 135 sera (68 men, 67 women). The age range was between 20 and 78 years. Pregnant women were excluded. The reference population was selected according to normal homocysteine values.

N	Median		Range (2.5 th -97.5 th percentile)	
	pg/mL	pmol/L	pg/mL	pmol/L
135	425	314	197-771	145-569

These values should only be used as guidelines.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute); 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
Sample	Mean	Repeatability		Intermediate precision	
		SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 1	176	8.86	5.0	12.7	7.2
Human serum 2	405	13.0	3.2	17.5	4.3
Human serum 3	960	19.7	2.1	31.0	3.2
Human serum 4	1230	27.4	2.2	46.4	3.8
Human serum 5	1940	40.9	2.1	72.6	3.7
PreciControl Varia1	447	12.2	2.7	18.6	4.2
PreciControl Varia2	934	20.2	2.2	38.4	4.1

cobas e 411 analyzer					
Sample	Mean	Repeatability		Intermediate precision	
		SD	CV	SD	CV
	pmol/L	pmol/L	%	pmol/L	%
Human serum 1	130	6.54	5.0	9.37	7.2
Human serum 2	299	9.59	3.2	12.9	4.3
Human serum 3	708	14.5	2.1	22.9	3.2
Human serum 4	908	20.2	2.2	34.2	3.8
Human serum 5	1432	30.2	2.1	53.6	3.7
PreciControl Varia1	330	9.00	2.7	13.7	4.2
PreciControl Varia2	689	14.9	2.2	28.3	4.1

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Mean	Repeatability		Intermediate precision	
		SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 1	176	5.84	3.3	9.14	5.2
Human serum 2	407	8.24	2.0	12.7	3.1
Human serum 3	1010	13.2	1.3	21.1	2.1

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Mean	Repeatability		Intermediate precision	
		SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 4	1230	19.8	1.6	28.8	2.3
Human serum 5	1890	29.8	1.6	41.5	2.2
PreciControl Varia1	448	7.16	1.6	15.3	3.4
PreciControl Varia2	917	12.0	1.3	27.8	3.0

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Mean	Repeatability		Intermediate precision	
		SD	CV	SD	CV
	pmol/L	pmol/L	%	pmol/L	%
Human serum 1	130	4.31	3.3	6.75	5.2
Human serum 2	300	6.08	2.0	9.37	3.1
Human serum 3	745	9.74	1.3	15.6	2.1
Human serum 4	908	14.6	1.6	21.3	2.3
Human serum 5	1395	22.0	1.6	30.6	2.2
PreciControl Varia1	331	5.28	1.6	11.3	3.4
PreciControl Varia2	677	8.86	1.3	20.5	3.0

Method comparison

a) A comparison of the Elecsys Vitamin B12 assay (calibrated with Vitamin B12 CalSet II; x) and the Elecsys Vitamin B12 II assay (calibrated with Vitamin B12 II CalSet; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 100

Passing/Bablok ²⁵	Linear regression
$y = 0.952x + 15.1$	$y = 0.957x + 11.6$
$\tau = 0.977$	$r = 0.999$

The sample concentrations were between 69 and 1890 pg/mL (51 and 1395 pmol/L).

b) A comparison of the Elecsys Vitamin B12 II assay (y) and a commercially available method (x) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 106

Passing/Bablok ²⁵	Linear regression
$y = 0.923x + 4.90$	$y = 0.881x + 27.6$
$\tau = 0.952$	$r = 0.993$

The sample concentrations were between 182 and 1797 pg/mL (134 and 1326 pmol/L).

c) A comparison of the Elecsys Vitamin B12 II assay on the cobas e 601 analyzer (y) and the Elecsys Vitamin B12 II assay on the cobas e 411 analyzer (x) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 117

Passing/Bablok ²⁵	Linear regression
$y = 1.01x - 2.77$	$y = 1.01x + 3.22$
$\tau = 0.933$	$r = 0.995$

The sample concentrations were between 56 and 1887 pg/mL (41 and 1393 pmol/L).

Analytical specificity

The following cross-reactivities were found, tested with vitamin B12 concentrations of 129 pg/mL and 550 pg/mL.

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Cross-reactant	Maximum concentration tested ng/mL	Cross-reactivity %
Cobinamide dicyanide	210	0.003

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see <https://usdiagnostics.roche.com> for definition of symbols used):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

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