Elecsys Active B12

cobas®

REF		Σ	SYSTEM
08717010190	08717010500	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1550
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 167

Intended use

Immunoassay for the in vitro quantitative determination of active vitamin B12 (holotranscobalamin) in human serum. The assay is used as an aid in the diagnosis and treatment of vitamin B12 deficiency.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Vitamin B12 is an essential water-soluble micronutrient that cannot be produced by the human body.[1,2,3] Three carrier proteins are involved in the transport and absorption of vitamin B12 (cobalamin): intrinsic factor, transcobalamin (TC) and haptocorrin (HC).[1] The majority (70-90 %) of total serum vitamin B12 circulating in the blood is bound to HC.[4,5] Approximately 20-30 % of total circulating vitamin B12 is bound to TC (known as holoTC).[4] Cellular uptake from the circulation requires the biologically active holoTC fraction.[4,5] This form is able to enter cells to exert the biological effects of vitamin B12 and is therefore referred to as "active vitamin B12".[4,6]

Vitamin B12 is essential for carbohydrate, fat and protein metabolism as well as nucleic acid synthesis.[2] It serves as a cofactor for enzymes central to 2 important metabolic reactions: the production of methionine from homocysteine and the production of succinyl-coenzyme A from methylmalonyl coenzyme A.[7] Therefore, vitamin B12 is vital for processes such as cell division, maintenance of the central nervous system, as well as the formation and regeneration of red blood cells.[2,8]

Deficiency in vitamin B12 can have major clinical implications and cause irreversible damage if left untreated.[1,7,9] The most common manifestations of vitamin B12 deficiency are hematologic disorders, particularly affecting the formation of erythrocytes (e.g. megaloblastic anemia),[7,9] and neurological disorders, such as demyelination of the spinal cord and cognitive decline.[7] Vitamin B12 deficiency has also been associated with bone disorders[10] and optic neuropathy, though these are less frequent.[9]

The most common causes of vitamin B12 deficiency are malabsorption due to pernicious anemia (autoimmune gastritis) or due to aging, insufficient dietary intake and other acquired defects in vitamin B12 metabolism.[7,3,11]

Given that holoTC is the active fraction of vitamin B12[6] and has a shorter circulating half-life than holoHC,[5] a number of publications suggest measurement of holoTC as a more diagnostically accurate and clinically relevant indicator of vitamin B12 deficiency than total serum vitamin B12.[3,6,11,12,13] Unlike total serum vitamin B12, holoTC is believed to be stable in pregnancy.[6,8,11] Furthermore, HoloTC was shown to be the best-performing indicator of vitamin B12 status in the elderly population, suggesting active vitamin B12 for first-line assessment of vitamin B12 deficiency.[12]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a biotinylated monoclonal anti-holotranscobalamin antibody and a monoclonal anti-transcobalamin antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the 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.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as ACTB12.

M Streptavidin-coated microparticles, 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-holotranscobalamin antibody~biotin, 1 bottle, 9 mL: Biotinylated monoclonal anti-holotranscobalamin antibody (mouse) 2 mg/L; HEPES[b)] buffer 50 mmol/L, pH 6.8; preservative.

R2 Anti-transcobalamin antibody~$Ru(bpy)_3^{2+}$, 1 bottle, 9 mL: Monoclonal anti-transcobalamin antibody (mouse) labeled with ruthenium complex 0.5 mg/L; HEPES buffer 50 mmol/L, pH 6.8; preservative.

b) HEPES = [4-(2-hydroxyethyl)-piperazine]-ethane sulfonic acid

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

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:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

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

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

Elecsys Active B12

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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
on the analyzers	48 days

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.

Stable for 5 days at 15-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). The samples may be frozen 5 times.

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.

Do not use samples and controls stabilized with azide.

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

Due to possible evaporation effects, samples and calibrators 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 07726350190, CalSet Active B12, for 4 x 1.0 mL
- REF 07713223190, PreciControl Active B12, for 4 x 3.0 mL
- REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **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

Additional materials for **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

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the WHO International Standard NIBSC (National Institute for Biological Standards and Control) code 03/178.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 8 weeks when using the same reagent lot
- after 7 days when using the same reagent kit on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Active B12.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.
When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in pmol/L.

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
Human serum albumin	≤ 7 g/dL

Criterion: For concentrations of 3-20 pmol/L the deviation is ≤ 2.0 pmol/L. For concentrations > 20-100 pmol/L the deviation is ≤ 10 %. For concentrations > 100 pmol/L the deviation is ≤ 15 %.

There is no high-dose hook effect at holotranscobalamin concentrations up to 1000 pmol/L.

Pharmaceutical substances
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Mutations or polymorphisms in genes related, peripheral to, or even unrelated to the vitamin B12 metabolic pathway, can affect the results of the vitamin B12 tests.[10] In particular, recent case reports have shown that genetic variations in the transcobalamin gene (TCN2) can affect HoloTC test results and indicate falsely low levels of HoloTC.[14,15]

Limits and ranges

Measuring range

3.0-150 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 3.0 pmol/L. Values above the measuring range are reported as > 150 pmol/L.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 2.0 pmol/L

Limit of Detection = 3.0 pmol/L

Limit of Quantitation = 5.0 pmol/L

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with an intermediate precision CV of ≤ 10 %.

Dilution

Samples with holotranscobalamin concentrations above the measuring range can be diluted with Diluent Universal 2. The recommended dilution is 1:2 (either automatically by the analyzers, or manually). The concentration of the diluted sample must be ≥ 65 pmol/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Note: Sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range. As Diluent Universal 2 may contain low levels of endogenous vitamin B12, it is recommended that linearity studies be performed using a known low analyte-containing serum pool. Samples outside the measuring range can be diluted 1:2 with Diluent Universal 2; the effect of endogenous vitamin B12 concentration is insignificant at these levels.

Expected values

Because differences may exist with respect to population and dietary status, it is recommended that normal ranges be determined by each laboratory over a suitable period of time and in a statistically significant number of samples before clinical significance is attached to the results.

The values shown below were performed on samples from an apparently healthy population, using the Elecsys Active B12 assay. The calculation is based on 214 sera (99 men, 115 women). The age range was between 20 and 79 years. Pregnant women were excluded. The reference population was selected according to normal homocysteine values.

N	Median	Range (2.5th-97.5th percentile)
	pmol/L	pmol/L
214	77.0	37.5-188

These values should only be used as guidance.

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 (EP05-A3) 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					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	6.02	0.093	1.5	0.266	4.4
Human serum 2	24.9	0.433	1.7	0.527	2.1
Human serum 3	52.3	0.970	1.9	1.31	2.5
Human serum 4	74.7	1.58	2.1	2.33	3.1
Human serum 5	143	3.06	2.1	4.34	3.0
PC[c)] Active B12 1	25.9	0.688	2.7	0.832	3.2
PC Active B12 2	54.3	1.11	2.0	1.56	2.9

c) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	5.77	0.121	2.1	0.380	6.6
Human serum 2	24.1	0.363	1.5	0.610	2.5
Human serum 3	47.4	0.655	1.4	1.18	2.5
Human serum 4	62.4	0.971	1.6	1.58	2.5
Human serum 5	141	2.01	1.4	3.92	2.8
PC Active B12 1	23.8	0.417	1.8	0.493	2.1
PC Active B12 2	49.6	0.754	1.5	0.940	1.9

Elecsys Active B12

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Method comparison

a) A comparison of the Elecsys Active B12 assay, REF 07713207190 (y) with a commercially available method (x) using clinical samples gave the following correlations (pmol/L):

Number of samples measured: 289

Passing/Bablok[16]	Weighted Deming regression
y = 0.883x + 10.9	y = 0.963x + 8.59
τ = 0.787	r = 0.937

The sample concentrations were between 7.3 and 119 pmol/L.

b) A comparison of the Elecsys Active B12 assay, REF 08717010 190 (**cobas e** 411 analyzer; y), with the Elecsys Active B12 assay, REF 07713207190 (**cobas e** 411 analyzer; x), gave the following correlations (pmol/L):

Number of samples measured: 161

Passing/Bablok[16]	Linear regression
y = 0.974x - 4.10	y = 0.960x - 3.15
τ = 0.904	r = 0.986

The sample concentrations were between 7.58 and 150 pmol/L.

Analytical specificity

The following cross-reactivities were found, tested with active B12 concentrations of approximately 7 pmol/L, 48 pmol/L and 124 pmol/L.

Cross-reactant	Concentration tested pmol/L	Cross-reactivity %
Apotranscobalamin	500	n. d.[d)]
Haptocorrin	5000	n.d.

d) n. d. = not detectable

References

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets 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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

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

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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