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Vitamin B12

cobas®

Vitamin B12

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
04745736 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Binding assay for the in vitro quantitative determination of vitamin B_{12} in human serum and plasma.

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

Summary

Nutritional and macrocytic anemias can be caused by a deficiency of vitamin B_{12}. This deficiency can result from diets devoid of meat and bacterial products, from alcoholism, or from structural/functional damage to digestive or absorptive processes (forms of pernicious anemia). Malabsorption is the major cause of this deficiency through pancreatic deficiency, gastric atrophy or gastrectomy, intestinal damage, loss of intestinal vitamin B_{12} binding protein (intrinsic factor), production of autoantibodies directed against intrinsic factor, or related causes.[1,2] This vitamin is necessary for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies will lead to megaloblastic anemia, and vitamin B_{12} deficiency results in irreversible central nervous system degeneration. Vitamin B_{12} or folate are both of diagnostic importance for the recognition of vitamin B_{12} or folate deficiency, especially in the context of the differential diagnosis of megaloblastic anemia.

Radioassays were first reported for vitamin B_{12} in 1961.[3,4] All utilize ^{57}Co-cyanocobalamin radiolabeled tracers and intrinsic factor for binding vitamin B_{12}. The various commercial assays differ in their free versus bound separation techniques and choice of specimen pretreatment. The presence of endogenous serum binding proteins for cyanocobalamin (transcobalamins including R-protein) and of immunoglobulins directed against intrinsic factor require that specimens are either boiled or treated at an alkaline pH to release the vitamin B_{12} and destroy the binding proteins. In the late 1970's, radioassays using serum binding proteins or partially purified intrinsic factor measured levels of vitamin B_{12} which exceeded those determined by microbiological methods. This was caused by the presence of the serum binding protein or R-proteins in the assay. R-protein specificity is poor compared to that of intrinsic factor and vitamin B_{12} analogs were being measured in addition to vitamin B_{12} itself.[5,6,7,8] Since that time, recommendations have been established for the use of highly purified intrinsic factor throughout the industry.

The Elecsys Vitamin B_{12} assay employs a competitive test principle using intrinsic factor specific for vitamin B_{12}. Vitamin B_{12} in the sample competes with the added vitamin B_{12} labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex[a)].

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

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (15 µL) with the vitamin B_{12} pretreatment 1 and pretreatment 2, bound vitamin B_{12} is released.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B_{12}-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 B_{12} labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor-vitamin B_{12} 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.

Reagents - working solutions

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

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~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 10 mL:
Ruthenium labeled porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.

R2 Vitamin B_{12}~biotin (black cap), 1 bottle, 8.5 mL:
Biotinylated vitamin B_{12} 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.
H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

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

Response:

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

P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER or doctor/physician.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Product safety labeling primarily 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 applied were FDA-approved 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.[9,10]

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	12 weeks
on the analyzers	5 weeks

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, K_3-EDTA and Li-heparin plasma. Li-heparin plasma tubes containing separating gel can be used.

When sodium citrate, sodium fluoride/potassium oxalate are used, the values obtained are 23 % lower than those obtained from serum.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 2 days at 2-8 °C, 2 months at -20 °C. Freeze once only. Protect from light.

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.

Do not use samples and controls stabilized with azide.

Vitamin B_{12} determinations should be performed on serum or plasma samples from fasting patients.

Note: Samples with extremely high total protein concentrations (e.g. patients suffering from Waldenström's macroglobulinemia) are not suitable for use in this assay, since they may lead to the formation of protein gel in the assay cup. Processing protein gel may cause a run abort. The critical protein concentration is dependent upon the individual sample composition. The formation of protein gel was seen in samples (spiked with human IgG or human serum albumin) having a total protein concentration > 160 g/L.

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 04572459190, Vitamin B_{12} CalSet II, for 4 x 1 mL
- REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- 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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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 Combimagazine M, 48 magazines x 84 reaction vessels 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

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.

MODULAR ANALYTICS E170, **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.

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Calibration

Traceability: This method has been standardized against the Vitamin B_{12} assay (REF 11820753).

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). Renewed calibration is recommended as follows:

- after 1 month (28 days) 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 Varia.

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.

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

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

Conversion factors: pmol/L x 1.36 = pg/mL

pg/mL x 0.738 = pmol/L

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (triglycerides < 17.1 mmol/L or < 1500 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

In vitro tests were performed on 54 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.

Limits and ranges

Measuring range

22.0-1476 pmol/L or 30.0-2000 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 22.0 pmol/L or < 30.0 pg/mL. Values above the measuring range are reported as > 1476 pmol/L or > 2000 pg/mL.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 22.0 pmol/L or 30.0 pg/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with vitamin B_{12} concentrations above the measuring range can be manually diluted 1:2 with Diluent Universal. The concentration of the diluted sample must be > 738 pmol/L or > 1000 pg/mL. After manual dilution, multiply the results by the dilution factor 2.

Note: Sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range. As Diluent Universal may contain low levels of endogenous vitamin B_{12}, 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; the effect of endogenous vitamin B_{12} 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 assays before clinical significance is attached to the results of these tests.

The values shown below were obtained in a study performed in 2005 in the USA and Germany:

Region	N	Median		Range (2.5th-97.5th percentile)	
		pmol/L	pg/mL	pmol/L	pg/mL
Europe	291	263	357	141-489	191-663
USA	178	342	463	156-698	211-946

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 duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	%
HS[b)] 1	142	192	8.34	11.3	5.9	14.6	19.8	10.3
HS 2	264	358	14.8	20.1	5.6	20.4	27.7	7.7
HS 3	660	894	21.5	29.1	3.3	29.6	40.1	4.5
HS 4	1199	1625	45.8	62.1	3.8	48.0	65.1	4.0
PC V[c)]1	345	467	13.7	18.5	4.0	21.5	29.2	6.3
PC V2	707	958	23.0	31.1	3.3	37.3	50.6	5.3

b) HS = human serum

c) PC V = PreciControl Varia

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	%
HS 1	140	190	5.63	7.63	4.0	7.20	9.75	5.1
HS 2	252	341	7.36	9.97	2.9	8.63	11.7	3.4
HS 3	633	858	13.9	18.8	2.2	18.3	24.8	2.9

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MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	%
HS 4	1120	1518	19.3	26.2	1.7	25.8	34.9	2.3
PC V1	326	442	8.34	11.3	2.5	10.3	14.0	3.2
PC V2	658	891	16.1	21.8	2.4	17.8	24.1	2.7

Method comparison

A comparison of the Elecsys Vitamin B_{12} assay (MODULAR ANALYTICS E170 analyzer; calibrated with Vitamin B_{12} CalSet; x) and the Elecsys Vitamin B_{12} assay (MODULAR ANALYTICS E170 analyzer; calibrated with Vitamin B_{12} CalSet II; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 101

Passing/Bablok[11]	Linear regression
y = 0.982x - 0.018	y = 0.968x + 5.77
τ = 0.977	r = 0.999

The sample concentrations were between approximately 49 and 1691 pg/mL (approximately 36 and 1248 pmol/L).

A comparison of the Elecsys Vitamin B_{12} assay (MODULAR ANALYTICS E170 analyzer; calibrated with Vitamin B_{12} CalSet II; x) and the Elecsys Vitamin B_{12} assay (Elecsys 2010 analyzer; calibrated with Vitamin B_{12} CalSet II; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 100

Passing/Bablok[11]	Linear regression
y = 0.997x - 4.17	y = 0.978x - 0.479
τ = 0.930	r = 0.994

The sample concentrations were between approximately 55 and 1609 pg/mL (approximately 41 and 1187 pmol/L).

Analytical specificity

The following cross-reactivity was found:

Cobinamide dicyanide	200 ng/mL	0.024 %

References

1 Herbert V. Drugs effective in megaloblastic anemias. In Goodman LS and Gilman A (eds): The Pharmacological Basis of Therapeutics, 5th Ed. MacMillan Co. 1975;1324-1349.

2 Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr 2009;89(2):693S-6S. Epub 2008 Dec 30.

3 Barakat RM, Ekins P. Assay of vitamin B12 in blood: A simple method. Lancet 1961;2(7192):25-26.

4 Rothenberg SP. Assay of serum vitamin B12 concentration using Co57-B12 and intrinsic factor. Proceedings of the Society for Experimental Biology and Medicine 1961;108:45-48.

5 Rothenberg SP, DaCosta M, Rosenberg BS. A radioassay for serum folate: Use of a two-phase sequential incubation, ligand-binding system. New Eng J Med 1972;285(25):1335-1339.

6 Gutcho S, Mansbach L. Simultaneous radioassay of serum vitamin B12 and folic acid. Clin Chem 1977;23:1609-1614.

7 Kolhouse JF, Kondo H, Allen NC, et al. Cobalamin analogues are present in human plasma and can mask cobalamin deficiency because current radioisotope dilution assays are not specific for true cobalamin. New Eng J Med 1978;299(15):785-792.

8 BIO RAD Quantaphase B-12/Folate Radioassay Instruction Manual, März 1995.

9 Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

10 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

11 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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.

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
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