Nutritional and macrocytic anemias can be caused by a deficiency of vitamin B₁₂. This deficiency can result from diets devoid of meat and bacterial products, from alcoholism, or from structural/functional damage to digestive 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 B₁₂ binding protein (intrinsic factor), production of autoantibodies directed against intrinsic factor, or related causes. This vitamin is necessary for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies will lead to megaloblastic anemia, and vitamin B₁₂ deficiency results in irreversible central nervous system degeneration. Vitamin B₁₂ or folate are both of diagnostic importance for the recognition of vitamin B₁₂ or folate deficiency, especially in the context of the differential diagnosis of megaloblastic anemia.

Radioassays were first reported for vitamin B₁₂ in 1961. All utilize 

\[ ^{57}\text{Co-cyanocobalamin} \text{ radiolabeled tracers and intrinsic factor for binding vitamin B}_{\text{12}} \]. 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 (transcobalamin including R-protein) and of immunoglobulins directed against intrinsic factor requires that specimens are either boiled or treated at an alkaline pH to release the vitamin B₁₂ and destroy the binding proteins. In the late 1970’s, radioassays using serum binding proteins or partially purified intrinsic factor measured levels of vitamin B₁₂ 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 B₁₂ analogs were being measured in addition to vitamin B₁₂ itself. Since that time, recommendations have been established for the use of highly purified intrinsic factor throughout the industry. The Elecsys Vitamin B₁₂ 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.

\[ \text{Tris(2,2’-bipyridyl)ruthenium(II)-complex (Ru(bpy)}^{2+} \]

Test principle

- 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. 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.

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 according to the European directive 89/39/ECC:

- PT2: C - CORROSIVE, R 34, S 26, S 37/39 (sodium hydroxide)
- Xn: Harmful by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
- PT2: Xn - HARMFUL, R 20/21/22, S 45 (sodium cyanide)

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 treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.

Avoid the formation of foam with 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 via the respective reagent barcodes.

Storage and stability

Store at 2-8°C. Use the Elecsys Vitamin B₁₂ 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 Elecsys 2010 and cobas e 411: 5 weeks
- on MODULAR ANALYTICS E170 and cobas e 601: 5 weeks

- 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT1</td>
<td>Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 0.1028 g/mL; stabilizer, pH 5.5</td>
</tr>
<tr>
<td>PT2</td>
<td>Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L</td>
</tr>
<tr>
<td>M</td>
<td>Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative</td>
</tr>
<tr>
<td>R1</td>
<td>Intrinsic factor-Ru(bpy)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</td>
</tr>
<tr>
<td>R2</td>
<td>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</td>
</tr>
</tbody>
</table>
**Vitamin B12**

**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 and K2-EDTA plasma. When sodium citrate, sodium fluoride/potassium oxalate are used, the values obtained are by 23% lower as compared to serum.

Na-heparin and K2-EDTA plasma. When sodium citrate, sodium fluoride/potassium oxalate are used, the values obtained are by 23% lower as compared to serum.

**Materials required (but not provided)**

- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips
- Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags

**Materials provided**

- Cat. No. 04572459, Vitamin B12 CalSet II, for 4 x 1 mL
- Cat. No. 04415299, PreciControl Anemia, for 2 x 2 mL each of PreciControl Anemia 1, 2 and 3
- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent or 2 x 2 L measuring cell cleaning solution
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution
- Cat. No. 03083871, Diluent Universal, 2 x 36 mL sample diluent
- Cat. No. 04836693, Elecsys Vitamin B12 CalCheck, 3 concentration ranges
- Cat. No. 04836693, Elecsys Vitamin B12 CalCheck, 3 concentration ranges
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M
- Cat. No. 03083871, Diluent Universal, 2 x 36 mL sample diluent
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags

**Calibration**

**Traceability:** This method has been standardized against the Elecsys Vitamin B12 assay (Cat. No. 11820753). Every Elecsys Vitamin B12 reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot.

The predefined master curve is adapted to the analyzer by the use of Elecsys Vitamin B12 CalSet II.

**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. if quality control findings are outside the specified limits

**Quality control**

For quality control, use Elecsys PreciControl Anemia 1, 2 and 3. Other suitable control material can be used in addition. Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every 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 limits.

**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 < 10 g/dL), lipemia (triglycerides < 17.1 mmol/L or < 1500 mg/dL), and biotin < 205 mmol/L or < 50 ng/mL. Criterio: Recovery within ± 10% of initial value. In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after 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 streptavidin and ruthenium can occur.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
Vitamin B12

Measuring range
22-1476 pmol/L or 30-2000 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 22 pmol/L or < 30 pg/mL. Values above the measuring range are reported as > 1476 pmol/L or > 2000 pg/mL.

Dilution
Samples with vitamin B12 concentrations above the measuring range can be manually diluted 1:2 with Elecsys 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 Elecsys Diluent Universal 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 Elecsys Diluent Universal; 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 assays before clinical significance is attached to the results of these tests. These values should only be used as a guideline. 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
Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards):11 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

<table>
<thead>
<tr>
<th>Region</th>
<th>N</th>
<th>Median</th>
<th>Range (2.5th-97.5th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pmol/L</td>
<td>pg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pmol/L</td>
<td>pg/mL</td>
</tr>
<tr>
<td>Europe</td>
<td>291</td>
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<tr>
<td>USA</td>
<td>178</td>
<td>342</td>
<td>463 156-698 211-946</td>
</tr>
</tbody>
</table>

These values should only be used as a guideline. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Analytical sensitivity (lower detection limit)
22 pmol/L or 30 pg/mL

The 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, within-run precision, n = 21).

Method comparison
A comparison of the Elecsys Vitamin B12 assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B12 CalSet; x) and the Elecsys Vitamin B12 assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B12 CalSet II; y) using clinical samples gave the following correlations (pg/mL):

- Number of samples measured: 101

Passing/Bablok12 Linear regression
\[ y = 0.982x - 0.018 \quad y = 0.968x + 5.77 \]
\[ t = 0.977 \quad r = 0.999 \]

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

A comparison of the Elecsys Vitamin B12 assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B12 CalSet II; x) and the Elecsys Vitamin B12 assay (Elecsys 2010 analyzer; calibrated with Elecsys Vitamin B12 CalSet II; y) using clinical samples gave the following correlations (pg/mL):

- Number of samples measured: 100

Passing/Bablok12 Linear regression
\[ y = 0.997x - 4.17 \quad y = 0.978x - 0.479 \]
\[ t = 0.930 \quad r = 0.994 \]

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

Analytical specificity
The following cross-reactivity was found:
Cobinamide dicynide 200 ng/mL 0.024%

References
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