Snibe Diagnostic

CE 0123 REF 130263002M:100 tests/kit
130663002M: 50 tests/kit

MAGLUMI® Vitamin B12 (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Vitamin B12 (Vit B12) in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis of patients with diseases related to Vitamin B12 deficiency.

SUMMARY

Vitamin B12 (commonly known as cyanocobalamin) is one of a group of complex molecules with a cobalt-containing corrin ring synthesized by microorganisms. The cobalt is located in the center of a ring-contracted modified tetrapyrrole macrocycle, coordinated via the four pyrrole nitrogen atoms[1,2]. Vitamin B12 is naturally found in foods including meat (especially liver and shellfish), eggs, and milk products[3]. When ingested, they are bound by a protein termed intrinsic factor in the gastric juice of the stomach and are subsequently absorbed in the ileum[4]. The transport of vitamin B12 in the blood as well as its tissue and hepatic uptake require the presence of transcobalamins (TCBs). Severe disorders are observed in congenital deficiencies in TCB II, illustrating the vital role played by this protein, including developmental neuropsychiatric disorders as well as pancytopenia-type haematological disorders and aregenerative megaloblastic anaemia[5].

The recognition and treatment of vitamin B12 deficiency is critical since it is a reversible cause of bone marrow failure and demyelinating nervous system disease. Insufficient intake or disrupted absorption of vitamin B12 will result in vitamin B12 deficiency. The most frequent cause of severe vitamin B12 deficiency is a loss of intrinsic factor due to autoimmune atrophic gastritis, historically called "pernicious anemia", even though many patients present with mainly neurologic manifestations[6,7]. Macrocytosis due to vitamin B12 or folate deficiency is a direct result of ineffective or dysplastic erythropoiesis. These abnormalities are caused by a defect in DNA synthesis that interferes with cellular proliferation and maturation. The erythroblasts become large, oval shaped and contain a characteristic immature, lacy nucleus. These bone marrow features are called "megaloblastic" and are highly suspicious of a vitamin B12 or folate deficiency[8].

Osteoporosis and related fractures represent major public health problems. Vitamin B12 has been associated with osteoblast activity and bone formation, and patients with pernicious anemia have been shown to have greater risk of fracture. Clinical studies have shown that vitamin B12-deficient patients have a higher risk of fracture[9].

Since the liver plays an important role in the storage and transport of cobalamin, it is not surprising that liver pathology is associated with major changes in plasma cobalamin concentrations. Acute hepatitis can hence be accompanied by high serum cobalamin in 25–40% of cases. In cirrhoses, the decrease in tissue and cellular liver uptake of vitamin B12 and of the haptocorrin–cobalamin complex are the main mechanisms involved and have been typified by biopsy studies performed in cirrhotic patients. Alcoholic liver diseases occupy an important place amongst high serum cobalamin cases stemming from liver disease[5,10].

The renal uptake of both folate and vitamin B12 involves glomerular filtration followed by tubular reabsorption. Significant amounts of vitamins are filtered daily, and because urinary excretion of B12 and intact folate is low, both are reabsorbed within the renal tubular system to prevent urinary loss. Furthermore, tubular uptake may result in kidney accumulation and possibly metabolism of B12. Kidney failure is among the causes to look for when confronted with high serum cobalamin. The suggested mechanism is serum accumulation of TCBs[5,11].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, Pretreatment Reagent 1 and Pretreatment Reagent 2 are mixed thoroughly and incubated. ABEI labeled with Vitamin B12 binding protein antigen and magnetic microbeads coated with Vitamin B12 immune complex antibody are then added and incubated. Vitamin B12 immune complex antibody coated on magnetic microbeads and antigen-antibody complex bound or ABEI form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of Vitamin B12 present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with Vitamin B12 immune complex antibody (~8.00 µg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	1.5 mL
Calibrator Low	A low concentration of Vitamin B12 antigen in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL
Calibrator High	A high concentration of Vitamin B12 antigen in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL
Pretreatment Reagent 2	NaOH (0.4%).	6.5 mL	4.0 mL
ABEI Label	ABEI labeled with Vitamin B12 binding protein antigen (~208 ng/mL) in PBS buffer, NaN_3 (<0.1%).	7.5 mL	4.5 mL
Pretreatment Reagent 1	PBS buffer, NaN_3 (<0.1%).	7.5 mL	4.5 mL
Control 1	A low concentration of Vitamin 312 antigen (150 pg/mL) in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL
Control 2	A high concentration of Vitamin B12 antigen (600 pg/mL) in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL
All reagents are provided ready-to-use.			

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- *Exercise the normal* precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.
- The safety data sheet is available on request.
- Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals

contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.

- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Do not transfer opened integral reagents between instruments.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive, or tubes containing clot activator or clot activator with gel
Plasma	K2-EDTA, Na-heparin or Li-heparin

- 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 types of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips are recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- For reliable results, specimens should be free of fibrin, red blood cells, or other particulate matter. Specimens must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 100 µL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 3 days at 10-30°C or 7 days at 2-8°C, or 2 months frozen at -20°C. Frozen specimens subjected to 1 freeze/thaw cycle have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, with Vitamin B12 concentrations above the analytical measuring interval. The recommended dilution ratio is 1:5(sample volume:total volume). The concentration of the diluted sample must be >400 pg/mL (295 pmol/L).
- For manual dilution, multiply the result by the dilution factor.

PROCEDURE

Materials Provided

Vitamin B12 (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000 Plus, MAGLUMI X3, MAGLUMI X6, MAGLUMI X8, MAGLUMI X10, or Integrated System Biolumi CX8.
- For Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip and Reaction Cup, please refer to corresponding Analyzer Operating Instructions for catalogue number.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are *totally resuspended* homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on registrating calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on registrating quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on registrating samples, refer to the sample section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the WHO International Standard 03/178.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 28 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[12].

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the Vitamin B12 assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

The first eight digits of the lot number on the controls must match those of the corresponding reagent kit. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Vitamin B12 (CLIA) Controls (REF: 1602011044MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Vitamin B12 concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in pg/mL or pmol/L. For further information please refer to the Analyzer Operating Instructions.

Conversion Factor: pmol/L×1.355=pg/mL; pg/mL × 0.738 = pmol/L

Interpretation of Results

The expected range for the Vitamin B12 assay was obtained by testing 594 apparently healthy individuals, gave the following expected value:

190-824 pg/mL (140-608 pmol/L) (2.5^{th}-97.5^{th} percentiles).

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the Vitamin B12 results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies[13,14]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[15].
- Bacterial contamination of the specimens may affect the test results.
- Since internal factors are often used as binding proteins in serum Vit B12 assails, anti-internal factor antibodies (which are common in pernicious anemia) can result in elevated Vit B12 measurements[16,17].

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (pg/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (pg/mL)	%CV	SD (pg/mL)	%CV	SD (pg/mL)	%CV
Serum Pool 1	150.548	6.060	4.02	4.578	3.04	12.512	8.31
Serum Pool 2	573.342	19.412	3.39	15.086	2.63	46.599	8.13
Serum Pool 3	1103.068	33.005	2.99	41.997	3.81	84.969	7.70
Plasma Pool 1	154.186	7.865	5.10	3.500	2.27	12.798	8.30
Plasma Pool 2	634.332	19.040	3.00	13.311	2.10	48.979	7.72
Plasma Pool 3	1137.326	34.666	3.05	24.631	2.17	82.686	7.27
Control 1	141.599	6.784	4.79	2.080	1.47	11.332	8.00
Control 2	579.523	15.110	2.61	15.399	2.66	44.644	7.70

Based on internal testing on the MAGLUMI and Biolumi systems, the overall repeatability (% CV) is estimated to be ≤ 5% for samples tested, the overall reproducibility (%CV) is estimated to be ≤ 10%. Performance of the assay at individual laboratories may vary.

Linear Range

60.0-2000 pg/mL (44.3-1476 pmol/L) (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

50.0-10000 pg/mL (36.9-7380 pmol/L) (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =30.0 pg/mL (22.1 pmol/L).

Limit of Detection (LoD) =50.0 pg/mL (36.9 pmol/L).

Limit of Quantitation (LoQ) =60.0 pg/mL (44.3 pmol/L).

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Hemoglobin	1000 mg/dL	Total protein	12 g/dL
Intralipid	4000 mg/dL	IgG	2.8 g/dL
Bilirubin	65 mg/dL	IgM	10 mg/mL
HAMA	40 ng/mL	IgA	16 mg/mL
ANA	398 AU/mL	Heparin sodium salt	80 IU/mL
Rheumatoid factor	1500 IU/mL	Heparin lithium salt	80 IU/mL
Biotin	0.5 mg/dL	K2-EDTA	22.75 µmol/mL

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactant in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to
Cobinamide dicyanide	200 ng/mL
Cobinamide	50 µg/mL
Folic acid	100 ng/mL

High-Dose Hook

No high-dose hook effect was seen for Vitamin B12 concentrations up to 10000 pg/mL (7380 pmol/L).

Method Comparison

A comparison of the Vitamin B12 assay with a commercially available immunoassay, gave the following correlations (pg/mL):

Number of samples measured: 129

Passing-Bablok: y=0.9846x+0.9250, r=0.943.

The clinical specimen concentrations were between 60.109 and 1999.822 pg/mL.

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SYMBOLS EXPLANATIONS

	Consult Instructions for use		Manufacturer
2°C-8°C	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for<n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit component
REF	Catalogue number	LOT	Batch code
CE 0123	CE marking with notified body ID number		

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