Snibe Diagnostic

CE

REF 130213002M: 100 tests
130613002M: 50 tests

MAGLUMI® Vitamin B12 (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Vitamin B12 in human serum using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system via the synthesis of myelin (myelinogenesis), and the formation of red blood cells. It is one of eight B vitamins. It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism[1-2]. No fungi, plants, or animals (including humans) are capable of producing vitamin B12. Only bacteria and archaea have the enzymes needed for its synthesis[3]. Some substantial sources of B12 include animal products (shellfish, meat), fortified food products, and dietary supplements. B12 is the largest and most structurally complicated vitamin and can be produced industrially through bacterial fermentation synthesis, typically used to manufacture B12 for fortified foods and supplements. It can also be produced synthetically via vitamin B12 total synthesis[4].

Vitamin B12 deficiency is most commonly caused by low intakes, but can also result from malabsorption, certain intestinal disorders, low presence of binding proteins, and use of certain medications. Vitamin B12 is rare from plant sources, so vegetarians are more likely to suffer from vitamin B12 deficiency. Infants are at a higher risk of vitamin B12 deficiency if they were born to vegetarian mothers. The elderly who have diets with limited meat or animal products are vulnerable populations as well. Vitamin B12 deficiency may occur in between 40% to 80% of the vegetarian population who are not also consuming a vitamin B12 supplement[5].

Vitamin B12 deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system[6]. At levels only slightly lower than normal, a range of symptoms such as fatigue, lethargy, depression, poor memory, breathlessness, headaches, and pale skin, among others, may be experienced, especially in elderly people (over age 60) who produce less stomach acid as they age, thereby increasing their probability of B12 deficiencies. Vitamin B12 deficiency can also cause symptoms of mania and psychosis[7-8].

PRINCIPLE OF THE TEST

The Vitamin B12 assay is a competitive chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), diluent, buffer are added and incubated, then add ABEI labeled with VB12-binding-protein, incubate, forming immuno complexes, then add magnetic microbeads coated with purified VB12 antigen and incubated. VB12 present in the sample (or calibrator/control, if applicable) competes with VB12 immobilized on the magnetic microbeads for a limited number of binding sites on the ABEI-labeled VB12-binding-protein. After precipitation in a magnetic field, decant the supernatant, then perform a wash cycle. Subsequently, the Starter 1+2 are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is inversely proportional to the concentration of VB12 present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material provided

Components	Contents	100 tests (REF: 130213002M)	50 tests (REF: 130613002M)
Magnetic Microbeads	Magnetic microbeads coated with VB12 antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and VB12 antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	Containing BSA and VB12 antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Buffer	0.4 mol/L NaOH.	15.0 mL	15.0 mL
ABEI Label	VB12 binding-protein labeled with ABEI, containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
Diluent	Phosphate buffer, NaN_3 (<0.1%).	15.0 mL	15.0 mL
Internal Quality Control	Containing BSA and VB12 antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
Sample Release Agent 1	DTT(30.0 mg, lyophilized)	1 bottle	1 bottle

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against the WHO 1st International Standard 03/178.

Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.

Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used (recommended).
- After instrument service is required.

- If controls lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.

Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***Vitamin B12 (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.

For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.

To monitor system performance and chart trends, commercially available quality control materials that covering at least two levels (high and low) of analyte is required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. 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 instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate substance.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed once. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details discussion of onboard sample storage constraints.
- The sample which has been placed at the room temperature more than 8 hours cannot be used again.
- Specimens removed from the separator, cells or clot may be stored up to 48 hours at 2-8°C.
- The sample serum with high concentration of protein (>90 g/L) cannot be used to do the tests. As the high dose of protein will form the gel and block the needle.
- Specimens can be stored up to 1 month frozen at -20°C or colder. Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of Vitamin B12 is 100 µL.

WARNING AND PRECAUTIONS FOR USERS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- CAUTION: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Stability for 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of Sample release agent 1

- The Sample release agent 1 is provided in a lyophilized form. The vial containing the lyophilized Sample release agent 1 must be opened carefully and reconstituted with 1 mL Diluent (D box).
- Cover the bottle stopper and gently shake to avoid producing bubbles.
- Allow the dissolved Sample release agent 1 to stand for 3 minutes.
- Transfer the dissolved Sample release agent 1 to the D box and slowly turn it up and down 10 times to make it well blended.
- After using, the kits including the dissolved Sample release agent 1 should be stored at 2-8 °C in an upright position.

Preparation of the Reagent

- 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.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

LIMITATIONS

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement.
- Any therapeutical decision should also be taken on a case-by-case basis.
- Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

Calculation of Results

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. For further information, please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

The expected ranges for the Vitamin B12 assay were obtained by testing 180 healthy individuals in China, and gave the following reference values listed below:
200-1100 pg/mL (2.5^{th}-97.5^{th} percentiles);
VB12 deficiency <200 pg/mL.
Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own expected ranges.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the Vitamin B12 assay was determined as described in the CLSI EP5-A2. 3 controls and 3 human serum pools containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(pg/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(pg/mL)	%CV	SD(pg/mL)	%CV	SD(pg/mL)	%CV
Serum Pool 1	200.440	11.779	5.88	7.652	3.82	14.046	7.01
Serum Pool 2	1102.935	35.533	3.22	24.883	2.26	43.379	3.93
Serum Pool 3	1499.221	40.090	2.67	26.767	1.79	48.204	3.22
Control 1	386.291	17.083	4.42	17.297	4.48	24.311	6.29
Control 2	511.972	19.890	3.89	7.074	1.38	21.192	4.14
Control 3	746.544	23.307	3.12	20.379	2.73	30.960	4.15

Limit of Blank (LoB)

The LoB for the Vitamin B12 assay is 12.5 pg/mL.

Measuring Range

12.5-2000 pg/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <12.5 pg/mL. Values above the measuring range are reported as >2000 pg/mL.

Recovery

The Vitamin B12 assay has a mean recovery of 90%-110%. Two different levels of Vitamin B12 were spiked into three samples resulted in the following data:

Sample	Added (pg/mL)	Observed (pg/mL)	%Recovery
S1	-	143.892	/
	65.11	208.742	99.60
	841.37	1000.827	101.85
S2	-	525.155	/
	65.11	591.632	102.10
	841.37	1340.442	96.90
S3	-	1045.848	/
	65.11	1110.633	99.50
	841.37	1890.584	100.40

Method Comparison

A total of 100 clinical samples in the range of 60.73 and 1999.31 pg/mL were tested by the Vitamin B12 assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=1.009x-4.008, r^2=0.985.

Analytical Specificity
The specificity of the assay was obtained by adding FA (100 ng/mL) to serum samples at the indicated concentrations. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:

- Bilirubin 20 mg/dL
- Hemoglobin 150 mg/dL
- Triglyceride 3000 mg/dL

REFERENCES

1. Miller, Ariel; Korem, Maya; Almog, Ronit; Galboiz, Yanina (June 15, 2005). "Vitamin B12, demyelination, remyelination and repair in multiple sclerosis". Journal of the Neurological Sciences. 233 (1-2): 93–97.
2. Yamada, Kazuhiro (2013). "Chapter 9. Cobalt: Its Role in Health and Disease". In Astrid Sigel, Helmut Sigel and Roland K. O. Sigel. Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences. 13. Springer. pp. 295–320.
3. Martens, J. H., Barg, H., Warren, M., & Jahn, D. (2002). Microbial production of vitamin B12. Applied microbiology and biotechnology, 58(3), 275-285.
4. Watanabe, F. (2007). Vitamin B12 sources and bioavailability. Experimental Biology and Medicine, 232(10), 1266-1274.
5. Pawlak, Roman; Parrott, Scott James; Raj, Sudha; Cullum-Dugan, Diana; Lucus, Debbie (1 February 2013). "How prevalent is vitamin B(12) deficiency among vegetarians?". Nutrition Reviews. 71 (2): 110–117.
6. Put, Nathalie M. J. van der; Straaten, Henny W. M. van; Trijbels, Frans J. M.; Blom, Henk J. (2001-04-01). "Folate, Homocysteine and Neural Tube Defects: An Overview". Experimental Biology and Medicine. 226 (4): 243–270.
7. Sethi, N. K., Robilotti, E., & Sadan, Y. (2005). Neurological manifestations of vitamin B-12 deficiency. The Internet Journal of Nutrition and Wellness, 2(1), 61-3.
8. Masalha R, Chudakov B, Muhamad M, Rudoy I, Volkov I, Wirguin I (2001). "Cobalamin-responsive psychosis as the sole manifestation of vitamin B12 deficiency". Israeli Medical Association Journal. 3 (9): 701–703.

Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax: +86-755-28292740

Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726

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		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code