

# MAGLUMI Vitamin B12 (CLIA)



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100



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**FOR PROFESSIONAL USE ONLY**  
Store at 2...8 °C



COMPLETELY READ THE INSTRUCTIONS BEFORE  
PROCEEDING



## SYMBOLS EXPLANATIONS



Authorized Representative in Europe



Manufacturer



Attention. See Instructions For Use



Contents of kit



In vitro diagnostic medical device  
(In vitro diagnostic use)



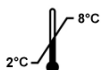
Lot number



Catalogue Code



Expiry date (Use by...)



Temperature limitation  
(store at 2...8 °C)



Number of tests



Keep away from sunlight



Keep upright

## INTENDED USE

The kit has been designed for the quantitative determination of Vitamin B12 in human serum.

The method can be used for samples over the range of 0-2000 pg/ml.

The test has to be performed on the MAGLUMI chemiluminescence immunoassay (CLIA) fully auto analyzer (Including MAGLUMI 1000, MAGLUMI 2000, MAGLUMI 2000 Plus and new developed models).

## SUMMARY AND EXPLANATION OF THE TEST

VB12 refers to a group of cobalt-containing vitamer compounds known as cobalamins, the main molecular form of 5-deoxyadenosine cobalamin. Cyanide cobalt amine (MW 1355) is the most stable, usually a reference for the determination of serum components.

Cobalamin can get from animal products such as meat, eggs, milk and other dairy products. During the intake process, cobalamin conjugate with a factor known as the combining-protein in the stomach, then absorbed by the ileum. Intrinsic factor is necessary for absorption process, once getting into the circulation; the cobalamin is stored in the liver. When being required, the cobalamin will be released into the plasma by the VB12 binding protein.

VB12 is normally involved in the metabolism of every cell of the body, especially affecting the DNA synthesis and regulation but also fatty acid synthesis and energy production. However, many (though not all) of the effects of functions of VB12 can be replaced by sufficient quantities of folic acid (vitamin B9), since VB12 is used to regenerate folate in the body. Most VB12 deficiency symptoms are actually folate deficiency symptoms, since they include all the effects of pernicious anemia and megaloblastosis, which are due to poor synthesis of DNA when the body does not have a proper supply of folic acid for the production of thymine.

The lack of VB12 can caused by a variety of reasons, the most common reason is a lack of intrinsic factor, resulting in that VB12 can not be absorbed from food. This condition is called anemia, mostly happening in people over the age of 50 years. the gastric resection is another reason of VB12 deficiency, because surgical resection and malabsorption caused by various bacteria or inflammatory disease affecting the small intestine. VB12 uptake has a positive correlation with the functional length of small intestine. The lack of VB12 is relatively rare caused by abnormal dietary, only if no consumption of animal's food in the next few years.

## PRINCIPLE OF THE TEST

Competitive immunoluminometric assay:

Use purified VB12 antigen to label ABEI, and use a VB12 binding-protein to label FITC. Sample, Calibrator or Control with ABEI Label, FITC Label and nano magnetic microbeads coated with sheep anti-FITC are mixed thoroughly and incubated at 37°C, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of VB12 present in controls or samples.

**CONT****KIT COMPONENTS****Material Supplies**

Reagent Integral for 100 determinations	
<b>Nano magnetic microbeads:</b> TRIS buffer, 1.2% (W/V), 0.2%NaN <sub>3</sub> , coated with sheep anti- FITC polyclonal antibody..	2.5ml
<b>Calibrator Low:</b> bovine serum, 0.2%NaN <sub>3</sub> .	3.0ml
<b>Calibrator High:</b> bovine serum, 0.2%NaN <sub>3</sub>	3.0ml
<b>Displacing Reagent</b> (preparation required)	
<b>FITC Label:</b> VB12 binding-protein labeled FITC, containing BSA, 0.2%NaN <sub>3</sub> .	13.5ml
<b>ABEI Label:</b> purified VB12 antigen labeled ABEI, containing BSA, 0.2%NaN <sub>3</sub> .	13.5ml
Prepare Displacing Reagent before using the integral	

Reagent Vials in kit box	
Empty bottle of displacing reagent	6 bottles
<b>NaOH Solution:</b> 1M NaOH	15ml
<b>DTT:</b> lyophilized 30mg DTT, reconstituted with 300µl distilled water	300µl
<b>Internal Quality Control:</b> containing BSA, 0.2%NaN <sub>3</sub> . (target value refer to Quality Control Information date sheet)	2.0ml

**Accessories Required But Not Provided**

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

**Preparation of the Reagent Integral**

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!) Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

**Do not interchange integral component from different reagents or lots!**

**Preparation of Displacing Reagent**

1. Use 300µl distilled water to dissolve the lyophilized DTT completely in DTT reagent vial, then aliquot into 3 empty tubes by 100µl each (Eppendorf tube, 0.5ml type is recommended). The dissolved DTT tube should be sealed and stored at -20°C and can be stable for 2 month. Take it out to room temperature before preparing Displacing Reagent.

2. Pipette 5ml NaOH into the empty displacing reagent bottle, then pipette 50µl DTT solution from DTT tube and add in the displacing reagent bottle. Using the NaOH solution in the bottle to rinse the wall, make sure DTT solution is dissolved with NaOH solution completely. Then horizontally shake the bottle in round cycles for mixing, avoided forming bubbles.

3. Place Displacing Reagent bottle into the 4<sup>th</sup> position of the kit integral. This bottle can be used for about 40 tests. (It is recommended customer collect enough samples and run the tests together).

**Note:**

- The prepared displacing reagent can only be used for 108 hours at 2-8°C. After 108 hours, the displacing reagent will be expired, and it should be discarded

- Displacing reagent shelf life will be shorted if place on the analyzer, it is recommended to finish the displacing reagent in 48 hours to ensure its performance.

**Storage and Stability**

- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.



- Keep upright for storage.



- Keep away from direct sunlight.

**CALIBRATION AND TRACEABILITY****1) Traceability**

To perform an accurate calibration, we have provided the test calibrators standardized against the Non WHO Reference Material Human Serum Vitamin B12, Pernicious anaemia 82/652

**2) 2-Point Recalibration**

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

**3) Frequency of Recalibration**

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the MAGLUMI Fully Auto analyzer.
- If controls are beyond the expected range.

**SPECIMEN COLLECTION AND PREPARATION**

Sample material: serum

Collect samples using standard procedures. Store at 2-8 °C: 24 hours, for longer storage periods: freeze to below -20 °C  
Avoid repeated freezing and thawing **more than 1 time**, stored samples should be thoroughly mixed prior to use (Vortex mixer).

**Vacuum Tubes**

- Blank tubes are recommended type for collecting samples.
- Please ask SNIBE for advice if special additive must be used in sample collecting.

**Specimen Conditions**

- The sample which has been placed at the **room temperature more than 8 hours cannot be used** again.
- The sample serum with **high concentration of protein (>160g/L) cannot be used** to do the tests. As the high dose of protein will form the gel and block the needle
- The Folic Acid is sensitive with light, please **avoid exposure to sunlight** when collect specimen
- Do not use specimens with the following conditions:
  - heat-inactivated specimens;
  - Cadaver specimens or body fluids other than human serum;
  - Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- 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 clot forms, the presence of fibrin may cause erroneous results.

**Preparation for Analysis**

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed discussion of onboard sample storage constraints.

**Storage**

- If testing will be delayed for more than 8 hours, remove serum or plasma from the serum or plasma separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 24 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

**Shipping**

- Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

**WARNING AND PRECAUTIONS FOR USERS**



- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. 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.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24, 2000, p.2143). A minimum of one hour at 121 °C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially

validating it and routinely using biological indicators.

- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens<sup>13</sup>. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

**Handling Precautions**

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

**TEST PROCEDURE**

To ensure proper test performance, strictly adhere to the operating instructions of the MAGLUMI Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

100µl	Sample , Calibrator
+100µl	Displacing reagent
2min	incubation
+110µl	ABEI label
+110µl	FITC label
+20µl	Nano magnetic microbeads
15 min	Incubation
400µl	Cycle washing
3 s	Measurement

**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 must be processed.

**QUALITY CONTROL**

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

**LIMITATIONS OF THE PROCEDURE**

**1) Limitations**

Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

A skillful technique and strict adherence to the instructions are

necessary to obtain reliable results.

Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results.

Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

## 2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin < 0.06 mg/ml, haemoglobin < 16 mg/dl or triglycerides < 12.5 mg/ml.

## 3) HAMA

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

### 1) Calculation of Results

- The analyzer automatically calculates the FA 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 MAGLUMI Chemiluminescence Analyzer Operating Instructions.

### 2) Interpretation of Results

- Results of study in clinical centers with group of individuals, 95% of the results were: 200-1100 pg/ml  
VB12 deficiency < 200 pg/ml
- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

## PERFORMANCE CHARACTERISTICS

### 1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

#### Intra-assay precision

Control	Mean(pg/ml)	SD(pg/ml)	CV%
Level 1	738.86	52.22	7.07%
Level 2	921.22	69.73	7.57%
Level 3	1103.06	85.24	7.73%

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

#### Inter-assay precision

Control	Mean(pg/ml)	SD(pg/ml)	CV%
Level 1	710.26	55.10	7.76%
Level 2	947.13	76.53	8.08%
Level 3	1125.68	95.44	8.48%

### 2) Analytical Sensitivity

The sensitivity is defined as the concentration of VB12 equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 50 pg/ml

### 3) Specificity

The specificity of the VB12 assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
FA	100 ng/ml	0.8%

### 4) Recovery

Consider calibrator high of known concentration as a sample,

dilute it by 1:2 ratio with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% -110%.

Expected	Mean Measuring	Recovery
630.561 pg/ml	641.732 pg/ml	101%

## 5) Linearity

Use VB12 calibrator to prepare the six-point standard curve, measuring all points' RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9800.

Calibrator Point	Concentration pg/ml	Absolute linear correlation coefficient (r)
A	0	
B	150	r=0.9910
C	300	
D	600	
E	1200	
F	2000	

## 6) Method comparison

A comparison of MAGLUMI VB12 (y) with a commercially available VB12 test (x) using clinical samples gave the following correlations (pg/ml):

Linear regression

$$y = 1.17x - 87.34$$

$$r = 0.967$$

$$S_{y,x} = 153.25$$

Number of samples measured: 190

The sample concentrations were between 151.2 and 1809 pg/ml.

## REFERENCES

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