

Instructions for Use

Vitamin B12 (CLIA)

[Product Name]

Vitamin B12 (CLIA)

[Packing Size]

24×1 Tests/Pkg (Calibrators included); 60×1 Tests/Pkg (Calibrators included); 60×1 Tests/Pkg

[Intended Use]

Vitamin B12 (VB12) assay is a chemiluminescent immunoassay (CLIA) used for the quantitative determination of Vitamin B12 (VB12) in human serum or plasma, and it is mainly used for auxiliary diagnosis of megaloblastic anemia.

Vitamin B12 is a corrinoid compound with four pyrrole rings around a cobalt atom, also known as cyanocobalamin. Vitamin B12 is a complex organometallic compound that is generally synthesized by microorganisms in nature and is water-soluble. Vitamin B12 in the human body can only be obtained from animal diets such as meat [1-3]. The absorption of vitamin B12 depends on intrinsic factor, a protein secreted by the parietal cells of the gastric mucosa. The combination of vitamin B12 and intrinsic factor attaches to the ileal mucosa, and is transported into the blood and tissues by transcobalamin. In addition to bone marrow and other tissues, most vitamin B12 is stored in the liver. There are many factors for vitamin B12 deficiency, among which the most common factor is intrinsic factor deficiency, which results in insufficient intake and absorption of vitamin B12 from food. For example, the most frequent cause of severe vitamin B12 deficiency is a lack of intrinsic factor due to autoimmune atrophic gastritis. The disease is historically called "pernicious anemia", even though many patients mainly present with neurologic manifestations [4-6]. In addition, other causes of vitamin B12 deficiency include gastrectomy, malabsorption, inflammatory bowel disease, etc. Vitamin B12 and folate are essential for normal DNA synthesis. Of them, vitamin B12 is a coenzyme involved in two metabolic functions critical for normal cell growth and DNA synthesis: the synthesis of methionine and the conversion of methylmalonyl CoA to succinyl CoA. Due to vitamin B12 deficiency, DNA synthesis is blocked, resulting in macrocytic anemia. The main symptom of this anemia is abnormal maturation of red blood cell precursors in the bone marrow [7-10]. Vitamin B12 deficiency or depletion may have other effects, including increased risk of neural tube abnormalities, osteoporosis, cerebrovascular and cardiovascular diseases [11, 12]. Due to the insidious nature and the risk of permanent neurological damage of these diseases, measurement of vitamin B12 for early diagnosis are critical and highly recommended for prevention of these diseases.

[Principle of the Assay]

This VB12 immunoassay adopts a competition format. The test principle is as follows:

- (1) Sample, pretreatment reagent 1 (PT1), and pretreatment reagent 2 (PT2) are added to the reaction well, mixed and incubated;
- (2) Acridinium labeled intrinsic factor is added to the reaction well. After mixing and incubation, VB12 in the sample will bind to the intrinsic factor;
- (3) Transfer all solution mixture to react with magnetic microparticle coated with VB12 derivative. After incubation, free acridinium labeled intrinsic factor unbound by VB12 in the reaction mixture will bind to VB12 derivative coated microparticle, and form a complex;
- (4) A magnet captures the microparticle, and then unbound substance is washed off. Add pre-trigger and trigger solution to the reaction mixture sequentially to

initiate chemiluminescence reaction;

- (5) A photomultiplier tube is used to measure photons generated from the reaction. The count is inversely proportional to Vitamin B12 concentration in the sample. Concentration of Vitamin B12 is determined by an internal calibration curve.

[Main Components]

Packing Size

Component		Fill Volume		
		24×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg
VB12 Reagent Cartridge	Microparticle (R1)	24×50 μL	60×50 μL	60×50 μL
	Conjugate (R2)	24×100 μL	60×100 μL	60×100 μL
	Pretreatment reagent 1 (PT1)	24×50 μL	60×50 μL	60×50 μL
	Pretreatment reagent 1 (PT2)	24×25 μL	60×25 μL	60×25 μL
VB12 Calibrator	Vitamin B12 Calibrator C1	1×1.0 mL	1×1.0 mL	/
	Vitamin B12 Calibrator C2	1×1.0 mL	1×1.0 mL	/
Calibration Card		1 pcs	1 pcs	/

Main Composition

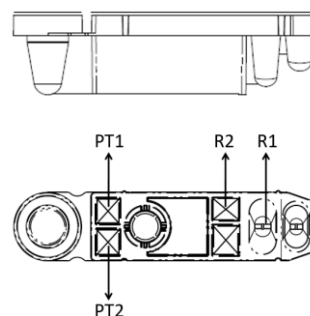
Component	Main Composition	
VB12 Reagent Cartridge	Microparticle (R1)	VB12 derivative coated microparticle, ~0.3 g/L; Tris buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Conjugate (R2)	Acridinium labeled intrinsic factor, ~100 μg/L; MES buffer, 50 mmol/L; ProClin 300, 0.5 g/L; Dicyanocobalaminamide, 2.0 mg/mL
	Pretreatment reagent 1 (PT1)	MES buffer, 50 mmol/L; DTT, 1.0 mg/mL
	Pretreatment reagent 2 (PT2)	NaOH solution, 0.5 mol/L
VB12 Calibrator C1	Vitamin B12; Tris buffer, 25 mmol/L; ProClin 300, 0.5 g/L	
VB12 Calibrator C2	Vitamin B12; Tris buffer, 25 mmol/L; ProClin 300, 0.5 g/L	
Calibration Card	Calibration curve and calibrator information	

Note: (1) Components in different lots of reagent cannot be mixed or exchanged for use.

(2) Traceability: This quantification method can be traced back to the WHO International Standard NIBSC Code: 03/178.

(3) Information about calibrators can be found in the instrument interface after scanning the Calibrator Card (such as lot number and concentration etc.)

The position of each component in the reagent pack is shown in the front view (Upper) and vertical view (Down) of the reagent cartridge.



Instruments and accessories needed but not included (available from Medcaptain)

- (1) Medcaptain Immu F6/F6S Automatic Chemiluminescent Immunoassay Analyzers;
- (2) Pre-trigger solution;
- (3) Trigger solution;
- (4) Washing solution;
- (5) 500 µL pipette tips;
- (6) Anemia Controls.

[Storage and Shelf-life]

Storage: Store sealed reagent cartridges and calibrators at 2~8°C in upright position, and avoid freezing.

Shelf life: 14 months.

Stability of Calibrators: Sealed vial of calibrators can be kept at 2~8°C in dark for 14 months. After calibrator C1 and C2 is uncapped and sealed again, it can be stored at 10~30°C for 5 days, and at 2~8°C for 60 days.

The dates of manufacturing and expiration can be found on the labels.

[Matched Instruments]

Medcaptain Immu F6/F6S Automatic Chemiluminescent Immunoassay Analyzers

[Specimen Types]

Serum, and plasma (collected with EDTA-K₂, EDTA-K₃, lithium heparin, and sodium heparin as the anti-coagulants) can be used in the testing.

Sample volume for each test: 20 µL.

The collected sample should be tested as soon as possible. **If the assay cannot be performed immediately, the sample should be kept protected from light.** Serum and plasma can be kept at 10~30°C for 24 hours, at 2~8°C for 3 days, and at -20°C or below for 60 days. Frequent freeze-thaw cycles should be avoided, and three freeze-thaw cycles are allowed. If the sample contains precipitate or frozen floccule, centrifugation is needed to clear the sample before testing.

Sample collection tubes from different manufacturers may lead to variation in testing results, due to the difference in tube material and additives in the tubes.

Medcaptain has not evaluated all types of sample collection tubes from different manufacturers. Each laboratory should make its own judgment about the usability of sample collection tubes.

[Test Procedure]

Reagent Preparation

Reagent: Vitamin B12 reagent cartridge (containing magnetic microparticle R1, acridinium labeled intrinsic factor conjugate R2, pretreatment reagent PT1, and pretreatment reagent PT2) is ready for use. It can be loaded directly into the instrument after opening the package.

Calibrators: Calibrator C1 and C2 are ready for use. Each calibrator can be added to a sample cup, and the cups are loaded to a sample rack. The sample rack can be pushed directly into the instrument for calibrator testing.

Calibration

Refer to the relevant chapter in the operation manual of each chemiluminescent immunoassay analyzer for system calibration. Calibration tests should be ordered before the first time use of Vitamin B12 assay. Medcaptain provides Vitamin B12 reagent pack and matched calibrators to calibrate the instrument.

Before calibration, scan the calibration card provided in the kit, and the calibration curve and calibrator information will be scanned into the system. Take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and

expiration date, etc.).

Put calibrators on a sample rack, load the sample rack into the instrument. On the screen interface of “Reagent > Request Calibration”, select test name and lot number to request a calibration. Select the position of each calibrator on the sample rack, set the number of repeat tests, start calibration.

Automatic immunoassay analyzer makes use of calibration data to validate the calibration, and adjust the calibration curve automatically.

Instrument calibration is effective for 60 days. A new calibration is needed in the following situation:

- (1) Change into a new lot of reagent;
- (2) Control test results are out of the target range;
- (3) The lot of reagent has been used on the same instrument for more than 60 days.

Refer to the Chapter of “Calibration” in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about calibration.

Control Testing

Anemia Controls are matched with Vitamin B12 reagent pack. There are two levels of controls: Low Control (L) and High Control (H).

Test two levels of controls with VB12 reagent on the instrument in accordance with any local applicable regulations. Control testing is highly recommended every time the lot of reagent has been changed, the instrument has been re-calibrated, or after trouble shooting/ maintenance service.

Before the control testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put controls on a sample rack, and load the sample rack into the instrument; Select “Control” on the interface of test menu, select test name and control lot; Click on “Start” and begin the testing. Check the results after control test is finished.

Control test results should fall into a specific range. If it is out of the target range, the user should check the system, such as expiration date of the controls, storage condition, instrument performance and status. After root cause analysis and corrective action, the user should test controls again. If the same problem exists, please contact customer service of Medcaptain.

Each laboratory should set up its own control range and frequency of control testing, based on its own practice. Refer to the Chapter of “Control Testing” in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about control testing.

Sample Testing

Before sample testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

If a sample collection tube is directly loaded to the instrument for testing, the sample volume should be at least 1.0 mL.

Un-cap sample collection tubes, put samples on a sample rack, and push the sample rack into the instrument; Select “Sample” on the interface of test menu, enter information of samples, select test name; Click on “Start” and begin the testing. Check the results after sample test is finished.

The reagent usage for each test is: R1 50 µL, R2 100 µL, PT1 50 µL, and PT2 25 µL. The instrument aspirates and mixes each component in the reagent cartridge, and incubates at 37°C. Time duration from sampling to result is about

30 min.

Refer to the Chapter of “Sample Testing” in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about sample testing.

Result Calculation

Based on the built-in calibration curve, the instrument automatically calculate Vitamin B12 concentration of each sample, either in a unit of pg/mL or pmol/L, 1.0 pg/mL= 0.738 pmol/L.

[Reference Intervals]

Samples for the study of reference intervals come from local area in Guangdong Province. A total of 240 healthy and normal people have been recruited (Male: 120; Female: 120), age ranges from 16 to 80 years old. Serum tests give a reference interval (2.5th-97.5th percentile) of 195~776 pg/mL, or 144~573 pmol/L.

Due to the differences in geography, race, sex, and age of tested population, the reference interval may vary in different laboratories. It is highly recommended for each clinical lab to establish its own reference intervals.

[Interpretation of Test Results]

The test data is for clinical reference only. It cannot be used as the only confirmatory evidence nor to eliminate the possibility of diseases. Clinical diagnosis of patients should take clinical symptoms, body sign, disease history, other lab test results, and treatment response into comprehensive consideration.

The measurement range of this assay is: 100~2000 pg/mL (73.8~1476 pmol/L). If Vitamin B12 concentration is lower than LoD, it will be reported as <100 pg/mL (<73.8 pmol/L); If Vitamin B12 concentration is over the upper limit, it will be reported as >2000 pg/mL (>1476 pmol/L). For a sample with Vitamin B12 concentration of >2000 pg/mL, sample diluent can be used to dilute the sample manually (a dilution factor of 1:2 is recommended). Test the diluted sample in duplicate to obtain more accurate results.

When the instrument shows a warning sign of “SMPL”, it means there is insufficient sample volume. Make sure enough sample is added for repeating the test. When the instrument shows a warning sign of “SMPJ”, it means the sample probe has been blocked. Clean sample clot in the probe before repeating the test. Some results are tagged with other signs. Refer to the Chapter of “Result Signs” in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about results tagged with signs.

[Limitation of the Test Method]

The test data is for clinical reference only. It cannot be used individually as the evidence to confirm or eliminate the possibility of diseases. For each endogenous interference substance with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

Endogenous interference substance	Concentration of interference substance
Total Protein	≤12 g/dL
Bilirubin	≤20 mg/dL
Hemoglobin	≤500 mg/dL
Triglyceride	≤1500 mg/dL

For potential cross-reactant with concentration shown in the table below, the test result of Vitamin B12 is less than 100 pg/mL.

Cross-reactant	Concentration of Cross Reactant
Cobinamide dicyanide	≤210 ng/mL

Heterophilic antibodies in human serum may react with immunoglobulin in the reagent or sample, and interfere with immunoassay in vitro. More clinical or diagnostic information is needed to confirm disease diagnosis of patients. Some patients have frequent contact with animals, or have been treated or diagnosed with mouse monoclonal antibodies. They may have generated heterophilic antibodies. For example, some patients under monoclonal antibody treatment may have human anti-mouse antibodies (HAMA) in blood circulation, leading to false positive or false negative results. Anti-interference components are added to this reagent formulation to minimize the impact of HAMA and ANA, but the problem may not be totally eliminated, and some sample testing may still be impacted. More clinical and diagnostic information is needed to make a solid conclusion.

Samples with a titer of no less than 1:1000 by anti-nuclear IgG test kit (indirect immune-fluorescence method) were studied in interference tests. It has shown less than ±10% error in the test results. For RF at a concentration of less than 1500 IU/mL, and for multiple representative human HAMA samples, the measurement error caused by the interference is within ±10%.

[Property and Performance]

1 Limit of Blank

LoB ≤ 50 pg/mL (≤36.9 pmol/L).

2 Limit of Detection

LoD ≤ 100 pg/mL (≤73.8 pmol/L).

3 Accuracy

Accuracy should meet at least one of the following criteria:

- a) Relative Deviation: Take International Reference Material which can be used as reference samples for routine evaluation. The relative deviation between the measurement result and the target value should not exceed ±10.0%.
- b) Relative Deviation: test the accuracy reference samples at two concentration levels multiple times. Relative deviation between the measurement result and the target value must not exceed 10.0%.
- c) Spike Vitamin B12 of a known concentration into real samples at different levels of Vitamin B12. Spiked recovery should be 100±5%.

4 Linearity

Test Vitamin B12 samples with concentration in the range of 100~2000 pg/mL, the correlation coefficient r ≥ 0.990.

5 Repeatability

Coefficient of variation (CV) for the test results of low (200±50 pg/mL) and high (550±150 pg/mL) corporate reference sample of VB12 is less than 8.0%.

6 Lot-to-lot Variation

Coefficient of variation (CV) for the test results of low (200±50 pg/mL) and high (550±150 pg/mL) corporate reference sample of VB12 with three batches of reagent is less than 10.0%.

7 Accuracy of Calibrator Value Assignment

Use primary calibrators with assigned values from higher level measurement procedure, calibrate the immunoassay analyzer, and use the same lot of reagent to measure the value of each product calibrator. The measured value of Calibrator C1 or C2 has a relative deviation within ±10.0% from its assigned value.

8 Homogeneity of Calibrators

8.1 Within-vial Homogeneity

Within-vial homogeneity of Calibrator C1 or C2 is represented with coefficient of variation, and CV ≤ 8.0%.

8.2 Between-vial Homogeneity

Between-vial homogeneity of calibrator C1 or C2 is represented with coefficient

[Attention Notes]

- 1 It is for in-vitro diagnosis only.
- 2 It can only be used by professionals.
- 3 Never use expired reagent kit.
- 4 Never mix components from different kits, or from different reagent lots.
- 5 Do not put the reagent cartridges upside down.
- 6 Measurement of Vitamin B12 in a sample using different detection systems may yield different results, due to the difference in test methods, assay specificity, and factors of interference. The measured values from different systems should not be directly compared to avoid inappropriate clinical interpretation.
- 7 Strictly follow the protocol in the package insert, and operate according to the lab guidelines.
- 8 The test results can only be used for clinical reference. Clinical diagnosis of patients should take symptoms, body sign, disease history, other laboratory test results, and response to treatment for comprehensive assessment.
- 9 User should wear gloves, safety goggles, and lab coat. Rinse with water if skin is in contact with the reagent. Flush eyes with copious of water if eyes are in touch with the reagent, and see a doctor immediately.
- 10 Take all samples and reaction waste as potential biohazard. All waste must be handled following the local government regulation.
- 11 This product is a single-use cartridge. Reagent cartridges should be put back into refrigerator and stored at 2~8°C if they have been placed at room temperature but not opened yet.

[Interpretation of Signs]

	Temperature limit.		Date of manufacturing
	<i>In vitro</i> diagnostic medical device		Catalogue number
	Batch Code		Consult instructions for use or consult electronic instructions for use
	Use-by Date		Authorized representative in the European Community/European Union
	This way up		CE marking
	Manufacturer		Unique device identifier
	Biological risks		

[References]

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[Basic Information]



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