



Vitamin B12 (CLIA) PACKAGE INSERT



INTENDED USE

Vitamin B12 (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of circulating Vitamin B12 (VB12) in human serum or plasma, which is intended as an aid to diagnosis of VB12 deficiency related diseases. The assay can be used for samples over the range of 125 pg/mL~2000 pg/mL. The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120 and other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Vitamin B12 is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement and a prescription medication. Vitamin B12 exists in several forms and contains the mineral cobalt, so compounds with vitamin B12 activity are collectively called “cobalamins”. Methylcobalamin and 5-deoxyadenosylcobalamin are the forms of vitamin B12 that are active in human metabolism. Vitamin B12 is required for proper red blood cell formation, neurological function, and DNA synthesis. Vitamin B12 functions as a cofactor for methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase catalyzes the conversion of homocysteine to methionine. Methionine is required for the formation of S-adenosylmethionine, a universal methyl donor for almost 100 different substrates, including DNA, RNA, hormones, proteins, and lipids. L-methylmalonyl-CoA mutase converts L-methylmalonyl-CoA to succinyl-CoA in the degradation of propionate, an essential biochemical reaction in fat and protein metabolism. Succinyl-CoA is also required for hemoglobin synthesis.

ASSAY PRINCIPLES

The principle of Vitamin B12 (CLIA) hereinafter also referred to as the “VB12” combines a competitive immunoassay method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as following: Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridges. All the assay steps are performed automatically by the ACCRE analyzer. The sample is manually pipetted into the sample well of reagent cartridge, then sample, pre-treatment solution 1 and pre-treatment solution 2 are mixed together, incubating at 37 °C, the pre-treatment solutions served as a displacing reagent to release the Vitamin B12 from its binding protein. Then samples are mixed with ALP swine intrinsic factor, incubating at 37 °C, the VB12 contains in the sample and intrinsic factor bonded together as an antibody-antigen immuno-complex. After 2nd incubation, VB12 coated magnetic particles are added into the immuno-complex, incubating at 37 °C for the 3rd time, the ALP labeled intrinsic factor bond to the VB12 coated magnetic particles, Then the mixture is washed in the magnetic field to eliminate the unbound components. The substrate (APS-5) is added to trigger the chemiluminescent reaction, the resulting chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is inversely proportional to the luminescence intensity. At the end, the results are automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls

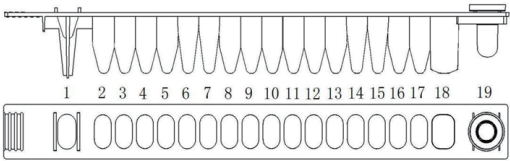
The calibration of this kit has been traced to Roche VB12, which can be traced to WHO Standard 03-178.

Contents	Quantities				Notice
	W118A	W118C	W05A	W05C	
VB12 Reagents Cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
VB12 Calibrator 1 (CAL1)	1.5 mL x 1 vial	1.5 mL x 1 vial	1.5 mL x 1 vial	1.5 mL x 1 vial	Tris Buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2g/L
VB12 Calibrator 2 (CAL2)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris Buffer,50mM; ProClin300, 0.5g/L; BND, 0.2g/L; Vitamin B12, 250pg/mL
VB12 Calibrator3 (CAL3)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris Buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2g/L; Vitamin B12, 1000pg/mL
VB12 Quality Control Level 1 (QC1)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Tris buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2 g/L; Vitamin B12, 300pg/mL Only for the configuration with Quality Controls
VB12 Quality Control Level 2(QC2)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Tris buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2 g/L; Vitamin B12, 1000pg/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Controls

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

Reagent Cartridge

The strip consists of 19 wells. The 1st well is the sample well and the last well 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a label, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridge. And please do not interchange integral component from different reagents or lots.



Description of The Reagent Cartridge

Well	Component	Content	Volume
2	Pre-treatment Solution1 (PT1)	Sodium Citrate,50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L; DTT, 1.024g/L	50μL
3	Pre-treatment Solution2 (PT2)	NaOH, 0.5mol/L	50μL
5	Enzyme Label (R2)	ALP (alkaline phosphatase) labeled swine intrinsic factor, 3μg/mL; PBS Buffer, 200mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	75μL
7	Magnetic Particles (R1)	VB12 coated magnetic particles, 0.2g/L; Tris Buffer, 50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	50μL
10, 11, 12,13	Wash Solutions	Tris Buffer, 50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	350μL
16	Substrate	APS-5	200μL

Note: Well 1,4,6,8,14,15,17,18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc)
- Pipette with disposable tip to dispense 200 μL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzer

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- 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.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Vitamin B12 (CLIA) kits including reagent cartridges, calibrators and quality controls upright at 2~8°C in dark place and the kit can be valid for 18 months from manufactured date.
- After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- When opened, calibrators and quality controls will be stable for 30 days at 2~8°C.
- Keep away from sunlight.
- Do not freeze reagent cartridges

SAMPLES

The sample volume for Vitamin B12 (CLIA) assay is 50μL, please note that at least 75 μL of sample must be pipetted manually into the reagent cartridge.

Sample Type and Sample Collection

Human serum or plasma (Heparin lithium, Heparin sodium, EDTA-K₂ or EDTA-K₃).

Types of tubes:

- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin lithium,
- Plastic tube with Heparin sodium,
- Plastic tube with EDTA-K₂.
- Plastic tube with EDTA-K₃.

It is recommended to validate collection tubes before use as some may contain substances which interfere with test results.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.

It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer’s recommendations for use.

Sample Preparation

The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.

For use of the sample tubes, follow the tube manufacturer’s instructions for use.

The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager. Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.

Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.

If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.

Severe hemolysis samples (hemoglobin >400mg/dL) or heat-inactivated samples are not recommended for applying on the reagents.

Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.

Mix using a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample Stability

Samples (serum and plasma) can be stored at 15~30°C for up to 4 hours or at 2~8°C for up to 3 days; if longer storage is required, freeze the serum or plasma at -20°C or below -20°C for up to 2 months, without exceeding 1 freeze/thaw cycle.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.

When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.

When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer.

Calibration is necessary under the following conditions:

- (1) New reagent lot number applied;
- (2) **Same lot of reagents used more than 4 weeks;**
- (3) Quality control out of control;
- (4) When necessary, for example: system maintenance or reipairement that may affect the analytical performance.

Test Procedure

- Remove the kit from storage at 2-8°C and take out the required reagent cartridge.
Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
- Use each assay VB12 strips for each sample, calibrator or control respectively.
- Place the reagent cartridge into the reagent rack.
- Manually pipette Sample, Calibrator, or Controls into the sample well of each reagent cartridge.

Please note that at least 75µL of sample has to be pipetted manually into the reagent cartridge and 50µL sample is needed for each test.

****Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.***

- Put the sample rack into the reagent chamber of ACCRE analyzer.
- Place the assay tips into the reagent chamber of ACCRE analyzer.
- Input Sample ID, sample type, dilution etc. into the software as directed by the USER MANUAL.
- Vitamin B12 (CLIA) assay will be completed within approximately 35 minutes. Assays will be performed automatically by the ACCRE analyzer.
- After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 2000pg/mL needs to be diluted manually. The recommended dilution is 1:10. And the concentration after dilution should be more than 200pg/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality Control

Quality control can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by ACCRE analyzer using stored calibration curve and calculated according to a predefined mathematical model. The results are expressed in pg/mL. The results can be converted as the unit of pg/mLor pmol/L by manually through below conversion factors.
Conversion Factor: pg/mL×0.738=pmol/L; pmol/L×1.36=pg/mL.

Interpretation of Results

The detection range is from 125pg/mL to 2000pg/mL. For samples with VB12 below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <125pg/mL; if the sample is higher than the upper limit of detection, the reported result is >2000pg/mL.

Results of Vitamin B12 (CLIA) assays are determined by testing specimens from a study in clinical centers with group of totally 202 healthy adult individuals in China, the reference rage determine by the range 2.5th percentile value and 97.5th percentile value is 204~831 pg/mL.

**** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.***

In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

LIMITATIONS

- If the test results are inconsistent with clinical evidences, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be interpreted together with other information; e.g., symptoms, results of other tests, clinical expressions, etc.

- Please note that heterophile antibody in human serum or plasma can interfere the test result.
- High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limits

LOB (Limit of Blank) = 50 pg/mL.
LoB values were determined according to CLSI® EP17-A2 recommendations.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).

The measurement range of the Vitamin B12 (CLIA) assay is 125 pg/mL to 2000 pg/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The Vitamin B12 (CLIA) assay is linear between 125 pg/mL to 2000 pg/mL. The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision

A precision study was performed according to CLSI® EP05-A3 recommendations. The within-lot precision CV% is not greater than 10%
The between-lot precision CV% is not greater than 12%

Interfering Substances

Potential interference substances were studied according to CLSI® EP07-A2 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤400mg/dL
Bilirubin	≤65mg/dL
Triglyceride	≤1500mg/dL
Total Protein	≤10g/dL
Rheumatoid factors	≤1500IU/mL

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 results¹¹⁻¹³.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	In vitro diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking
	Upright		Fragile, handle with care		Do not roll
	Control				

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