en

# Immunoassay

REF **CMS0301 / CMS0302 / CMS0303 / CMS0304 / CMS0305**

***50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2***

# Vitamin B12 CLIA Microparticles

*This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of Vitamin B12 concentration in human serum.*



**Key to Graphical Symbols Used**

| | | | |
|---|---|---|---|
| LOT | batch code | | use by |
| | manufacturer | Σ | contains sufficient for <n> tests |
| IVD | *in vitro* diagnostic medical device | | temperature limitation |
| REF | catalogue number | | consult instructions for use |
| EC REP | authorized representative in the European Community | | |

EC REP

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CE IVD

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## Introduction

Vitamin B12, also called cobalamin, is a water-soluble vitamin that is involved in the metabolism of every cell of the human body.[1] Vitamin $B_{12}$ has the largest and most complex chemical structure of all the vitamins. Vitamin $B_{12}$ or cobalamin plays essential roles in folate metabolism and in the synthesis of the citric acid cycle intermediate, succinyl-CoA.[2] Vitamin $B_{12}$ deficiency is commonly associated with chronic stomach inflammation, which may contribute to an autoimmune vitamin $B_{12}$ malabsorption syndrome called pernicious anemia and to a food-bound vitamin $B_{12}$ malabsorption syndrome. Impairment of vitamin $B_{12}$ absorption can cause megaloblastic anmia and neurologic disorders in deficient subjects.[3] Other causes of vitamin $B_{12}$ deficiency include surgical resection of the stomach or portions of the small intestine where receptors for the IF-$B_{12}$ complex are located.[4]

## Measurement Principle

This assay is based upon the one-step competitive method. The sample, intrinsic factor antibody coated microparticles, enzyme labeled intrinsic factor antibody are combined. The sample was pretreated with Pre-treatment Reagent A, Pre-treatment Reagent B, and Pre-treatment Reagent C to release the vitamin B12 in the sample. During the incubation, the vitamin B12 present in the sample and intrinsic factor antibody coated in the microparticles compete for binding to the Intrinsic Factor, a complex is generated between the solid phase, Intrinsic Factor and enzyme-linked intrinsic factor antibody by immunological reactions. After washing, the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is inversely proportional to the amount of vitamin B12 in the sample.

## Materials Provided

1. Calibrators

6 vials each containing 1.0 mL of Calibrator A through F. The matrix is citrate buffer containing BSA. Contains a selection of preservatives.

2. Reagent pack

Reagent pack provided ready to use.

| Σ | 50*1 | 100*1 | 100*2 | 100*5 | 50*2 |
|---|---|---|---|---|---|
| Microparticles Solution | 1.2mL*1 | 2.3mL*1 | 2.3mL*2 | 2.3mL*5 | 1.2mL*2 |
| Enzyme Conjugate | 3.0mL*1 | 5.5mL*1 | 5.5mL*2 | 5.5mL*5 | 3.0mL*2 |
| Intrinsic Factor | 2.3mL*1 | 3.5mL*1 | 3.5mL*2 | 3.5mL*5 | 2.3mL*2 |
| Pre-treatment Reagent A | 2.3mL*1 | 3.5mL*1 | 3.5mL*2 | 3.5mL*5 | 2.3mL*2 |
| Pre-treatment Reagent B | 2.3mL*1 | 3.5mL*1 | 3.5mL*2 | 3.5mL*5 | 2.3mL*2 |
| Pre-treatment Reagent C | 3.0mL*1 | 5.5mL*1 | 5.5mL*2 | 5.5mL*5 | 3.0mL*2 |

- Microparticles Solution

Contains of intrinsic factor antibody coated microparticles in Tris-HCl buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Contains of horseradish-peroxidase labeled intrinsic factor antibody in Tris-HCl buffer containing BSA. Contains a selection of preservatives.

- Intrinsic Factor

Contains of porcine intrinsic factor in Tris-HCl buffer containing and BSA. Contains a selection of preservatives.

- Pre-treatment Reagent A

Contains of NaOH.

- Pre-treatment Reagent B

Contains of acetic acid buffer.

- Pre-treatment Reagent C

Contains of boric acid buffer. Contains a selection of preservatives.

## Assay Analyzer on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

## Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needle
6. Wash Buffer used in washing procedure
7. Distilled or deionized water

## Metrological Traceability of Calibrators

The measurand (analyte) in the Assay Analyzer Vitamin B12 Calibrators is traceable to a higher order calibrator purchased from WHO (The World Health Organization) 03/178, at each concentration level.
The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

## Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. It is recommended all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hy-

pochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure microparticles are resuspended before loading on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.
16. Do not use reagents beyond the labeled expiry date.

## Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 1 months.

## Sample

1. Collect serum samples in accordance with correct medical practices. Light may cause accelerated degradation of folate and should be avoided in sample collection, transportation and storage.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
4. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
5. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Cap and store the samples at 18-25°C for no more than 6 hours. Or freeze the samples at 2-8°C for no more than 48 hours. For longer use, freeze the samples at -80°C, under which conditions the stability will be retained for 1 year. The sample can be frozen and thawed only once. Avoid strong light. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
8. Do not add substances into the sample which can change the PH.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

## Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample cups or tubes on the sample holder, 50 $\mu$L of samples for each test. But considering the sample container and 150 $\mu$L of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample holder and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Pre-treatment Reagent A and Pre-treatment Reagent B to the reaction vessel
  - Mix and incubate
  - Adds Pre-treatment Reagent C to the reaction vessel l
  - Adds Microparticles Solution, Intrinsic Factor and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of vitamin B12 in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample holder. Conduct duplicate detection on the system.
- Load the sample holder and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements

- A reagent kit and Chemiluminescent Substrate with new batch code is used
- Beyond the expiration date of a calibration curve
- Important parts of the analyzer are replaced or repaired

- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a Vitamin B12 value exceeding 2000 pg/mL may be diluted manually. Human serum with a low analyte concentration is used to dilute the samples. After dilution, multiply the result by the dilution factor.

## Measurement Results

The sample test results are determined automatically by the system software. The amount of vitamin B12 in the samples is determined from the measured light production by means of the stored calibration data. Sample test results can be reviewed using the appropriate screen. Refer to the Assay Analyzer's operation manual on reviewing sample results.
The default unit for this assay is pg/mL.
Conversion formula: pg/mL*0.738 = pmol/L; pmol/L*1.36=pg/mL

## Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

## Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. 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. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
5. Samples with extremely high total protein concentration (such as macroglobulinemia) may form gelatinous substances during sample processing, resulting in microparticles agglutination, clogging of reagent needles, etc., which not applicable to detection of this assay.
6. This test measures concentrations within the range of 100-2000 pg/mL. If Vitamin B12 concentrations above the measuring range to be expected, it is recommended to dilute samples with human serum with a low analyte concentration. The recommended dilution is 1:3 of this test, allowing samples to be up approximately 8000 pg/mL.

## Biological Reference Interval

Serum samples from 400 healthy people of different ages and genders in Henan province were tested. The 2.5% and 97.5% percentiles were used as the upper and lower limits of the reference interval, and the normal reference interval was determined to be 183 pg/mL – 822 pg/mL. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

## Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

| Panel Member | n | Mean (pg/mL) | Within-run | Total |
|---|---|---|---|---|
| | | | %CV | %CV |
| 1 | 80 | 266.19 | 4.33 | 8.14 |
| 2 | 80 | 462.69 | 4.28 | 5.88 |
| 3 | 80 | 1067.76 | 3.69 | 4.94 |

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank: 15 pg/mL.
Limit of Detection: 50 pg/mL.
Limit of Quantitation: 75 pg/mL with a coefficient of variation of 20%.

3. Analytical Specificity

Cross reaction: The following substances and concentrations were tested and found no cross reaction with the test.

| Substances | Concentration (ng/mL) | Cross reactivity % |
|---|---|---|
| Cobinamide | 200 | <50 pg/mL |

Interference: No interference with 30 mg/dL of Bilirubin, 3000 mg/dL of Triglyceride

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a Vitamin B12 reference assay. Data were analyzed and are summarized in the following table.

| Correlation Method | Number of Samples | Intercept | Slope | Correlation Coefficient |
|---|---|---|---|---|
| Linear Regression | 42 | 3.0717 | 1.089 | 0.9777 |

## Literature References

1. Yamada K."Chapter 9. COBALT: Its Role in Health and Disease". In Sigel A, Sigel H, Sigel RK. Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences. 13. Springer. pp. 295-320. 2013.

2. Brody T. Nutritional Biochemistry. 2nd ed. San Diego: Academic Press; 1999.

3. Carmel R. Cobalamin(Vitamin B12). In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, eds. Modern Nutrition in Health and Diease. Philadelphia: Lippincott Williams&Wilkins; 2006: 482-497.

4. Carmel R. How I treat cobalamin (vitamin B12) deficiency. Blood. 2008;112(6):2214-2221.