Vitamin B12

INTENDED USE
The Vitamin B12 ELISA kit is an enzyme immunoassay intended for the quantitative determination of vitamin B12 in human serum, plasma, tissue homogenates and other biological fluids.

SUMMARY
Vitamin B12, also called cobalamin, is a water-soluble vitamin that has a key role in the normal functioning of the nervous system via the synthesis of myelin (myelin genesis),(1, 2) and in the maturation of developing red blood cells in the bone marrow. It is involved in the metabolism of every cell of the human body: it is a cofactor in DNA synthesis, fatty acid metabolism, and amino acid metabolism.(3) Vitamin B12 deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system.(4) 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)(5, 6) who produce less stomach acid as they age, thereby increasing their probability of B12 deficiencies.(7) Vitamin B12 deficiency can also cause symptoms of mania and psychosis.(8)
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. (9) In Hong Kong and India, vitamin B12 deficiency has been found in roughly 80% of the vegan population as well.(10)

TEST PRINCIPLE
Delayed competitive assay. Total duration of assay: 115 minutes.
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody.
After a short incubation, the enzyme conjugate is added (this delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites (not consumed in the first incubation).
A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen con- centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS
Materials provided
• Coated Microplate, 96 wells, pre-coated with 10 µg/mL streptavidin.
• Calibrators, 5 vials, 0.8 mL each, ready to use; Concentrations: 0(A), 200(B), 400(C), 1000(D), and 2000(E) pg/mL.
• Enzyme Conjugate, 1 vial, 6.0 mL. Contains Vitamin B12 (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix
• Biotin Reagent, 1 vial, 6.0 mL. Contains anti-Vitamin B12 biotinylated purified rabbit IgG conjugate in buffer, dye and preservative.
• Substrate, 1 vial, 11 mL, ready to use, (tetramethylbenzidine) TMB.
• Stop Solution, 1 vial, 6.0 ml of 1 mol/l sulfuric acid.
• Wash Solution Concentrate, 1 vial, 25 mL (20X concentrated), PBS-Tween wash solution.
• Releasing Agent, 1 vial, 8 mL. Contains a strong base (sodium hydroxide) and potassium cyanide
• Stabilizing Agent, 1 vial, 5 mL. Contains tris 2-carboxyethyl phosphine (TCEP) solution.
• Neutralizing Buffer, 1 vial, 10 mL. Contains buffer with dye that reduces the pH of sample extraction.
• IFU, 1 copy.

MATERIALS REQUIRED (BUT NOT PROVIDED)
• Microplate reader with 450nm and 620nm wavelength absorbent capability
• Microplate washer.
• Incubator.
• Plate shaker.
• Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
• Absorbent paper.
• Distilled water.
• Plate Lid, 2 pieces.

PRECAUTIONS AND WARMINGS
• For in vitro diagnostic use only. For professional use only
• All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II. But all products should be reared as potential biohazards in use and for disposal.
• Mix the sample in the wells thoroughly by shaking and eliminate the bubbles
• Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
• Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
• Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
• Do not use reagents beyond the labeled expiry date.• Do not mix or use components from kits with different batch codes
• If more than one plate is used, it is recommended to repeat the calibration curve.
• It is important that the time of reaction in each well is held constant to achieve reproducible results.
• Ensure that the bottom of the plate is clean and dry.• Ensure that no bubbles are present on the surface of the liquid before reading the plate.
• The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

STORAGE
• Store at 2-8°C.• Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
• Seal and return unused calibrators to 2-8 °C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles.
• Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

SPECIMEN COLLETION AND PREPARATION
• Collect serum samples in accordance with correct medical practices.• Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 3 days at 2-8 °C, and 1 month at -20 °C. Recovery within 90-110 % of serum value or slope 0.9-1.1. Freeze only once.
• The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
• Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
• 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.
• Avoid grossly hemolytic, lipemic or turbid samples.• Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
• 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.
• Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
• Adjust the incubator to 37 °C
• Prepare wash solution before measurement. Stable for 2 months at ambient temperature.
• Don's use Substrate if it looks blue.
• Don't use reagents that are contaminated or have bacteria growth.

QUALITY CONTROL
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. There controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

REAGENT PREPARATION
Wash Solution: Dilute contents of Wash Solution Concentrate (25ml, 20X) to 500ml with distilled water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.

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Extraction agent : Obtain enough test tubes for preparation. Add **Stabilizing Agent(**2.7 ml) and
Releasing Agent(6.3 ml) to tubes as the ratio of 3:7, then mix thoroughly. should be prepared when using and do not keep mix for too long after prepared.
Sample extraction : Obtain enough test tubes for preparation of all patient samples, controls. Dispense 80µl of all samples into individual test tubes. Pipette 90µl of the prepared **extraction agent** to each test tube, shaking after each addition. Let the reaction proceed for 15 min, at 37°C. Then dispense 90µl of the **neutralizing buffer,** vortex. After the neutralizing buffer is added and mixed, let the reaction go to completion by waiting an additional 5 min (at 37°C)before dispensing into the **Coated Microplate.**

TEST PROCEDURE

• Use a pipette to aspirate 50ul of the calibration sample or sample that has been processed in the previous step, add it to the Coated Microplate, and then take 50ul of Biotin Reagent and add it to the micropores. Mix the microplate gently for 20-30 seconds to mix. Cover and incubate for 15 minutes at 37°C.
• Add 50 µL of Enzyme Conjugate to all wells. Mix the microplate gently for 20-30 seconds to mix. Cover and incubate for 15 minutes at 37°C.
• Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
• Add 350 µL of Wash solution. Repeat 4 additional times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
• Add 100 µL of Substrate reagent to all wells. Incubate at room temperature in the dark for 15 minutes. Do not shake the plate after substrate addition.
• Add 50 µL of Stop Solution to each well and gently mix for 30 seconds.
• Read the absorbance in each well at 450nm (using a reference wavelength of 620nm). The results should be read within 15 minutes of adding the stop solution.
Note: Dilute the samples suspected of concentrations higher than 2000 pg/mL 1:5 and 1:10 with Vitamin B12 '0' pg/mL calibrator and re-assay.
Do not use the working substrate if it looks blue. Do not use reagents that are contaminated or have bacteria growth. Use of multiple (3) touch vortex is recommended. It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the glass tubes at an angle while touching the side of the tubes.

CALCULATION

• Record the absorbance obtained from the printout of the microplate reader.
• Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation
• Plot the common logarithm of absorbance against concentration in pg/mL for each calibrator.
• Draw the best-fit curve through the plotted points on linear graph paper. 4- parameter logistic function curve fitting is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (pg/mL)	Absorbance
Calibrator A	0	2.427
Calibrator B	300	1.046
Calibrator C	550	0.527
Calibrator D	900	0.287
Calibrator E	1400	0.137

LIMITS AND RANGES

MEASURING RANGE

50-2000 pg/mL or 37-1476 pmol/L (defined by the Limit of Quantitation and the maximum of the master curve).
Values below the Limit of Quantitation are reported as < 50 pg/mL or ＜37 pmol/L.
Values above the measuring range are reported as > 2000 pg/mL or > 1476 pmol/L.

EXPECTED EVALUES

Population	pg/mL	pmol/L
Newborn	160 -1300	118 - 859
Adult	200 - 835	148 – 616
Adult > 60 years	110 - 800	81 - 590

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

SPECIFIC PERFOMANCE DATA

Representative performance data are given below. Results obtained in individual laboratories may differ.

PRECISION

Precision was determined using reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	**Mean (pmol/mL)**	**SD (pmol/mL)**	**CV (%)**	**SD (pmol/mL)**	**CV (%)**
Human Serum 1	245.2	25.1	10.2	27.2	11.1
Human Serum 2	562.1	34.3	6.1	36.3	6.5
Human Serum 3	802.3	26.1	3.3	30.4	3.8
PC Universal 1	305.8	15.9	5.2	22.8	7.5
PC Universal 2	825.6	46.2	5.6	57.4	7.0

*Repeatability = within-run precision

METHOD COMPARISON

A comparison of the vitamin B12 assay (y) with the Beckman Coulter ACCESS® Vitamin B12 (x) using clinical samples gave the following correlations:Number of samples measured: 67Linear regression
y = 1.0448x - 20.427
r = 0.9680

CODIGO: RSET106-3