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ACCESS
Immunoassay Systems

Instructions For Use

Access Vitamin B12 Cobalamin

REF 33000

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access Vitamin B_{12} assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of vitamin B_{12} levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

SUMMARY AND EXPLANATION

Vitamin B_{12} is the name given to any one of a group of substances termed cobalamins. They are composed of a tetrapyrole ring surrounding a central cobalt atom and differ with respect to the side groups attached to the cobalt atom. The predominant form in serum is methylcobalamin while the predominant cellular form is 5' deoxyadenosylcobalamin.[1] Cyanocobalamin (MW 1355) is the most stable and is used as a reference compound for measuring serum cobalamin concentrations.

Cobalamins are obtained from animal products such as meat, eggs, milk, and other dairy products. When ingested, they are bound by a protein termed intrinsic factor in the gastric juice of the stomach and are subsequently absorbed in the ileum. Intrinsic factor is required for absorption. Once in circulation, cobalamins are taken up and stored in the liver. They are released into the plasma as needed where they are carried by B_{12} binding proteins (transcobalamins).[1]

Vitamin B_{12} is a coenzyme that is involved in two very important metabolic functions vital to normal cell growth and DNA synthesis: 1) the synthesis of methionine, and 2) the conversion of methylmalonyl CoA to succinyl CoA. Deficiency of this vitamin can lead to megaloblastic anemia and ultimately to severe neurological problems.[2,3] Megaloblastic anemia is characterized by the enlargement and reduction in number of all rapidly proliferating cells of the body, including marrow cells, and is primarily a result of the decreased capacity for DNA synthesis. Because vitamin B_{12} and folic acid are linked by the reaction pathway for methionine synthesis, a deficiency in either will disrupt this metabolic pathway and lead to the same symptoms and medical problems. It is usually necessary to measure both vitamins in a clinical workup, with the treatment depending on which of the two is deficient.

Vitamin B_{12} deficiency can occur for one of several reasons.[1,2,4] The most common cause is a defect in the secretion of intrinsic factor, resulting in inadequate vitamin B_{12} absorption from foods. This condition is called pernicious anemia and is most common in people over age 50.[3] Other causes of vitamin B_{12} deficiency are gastrectomy, malabsorption due to surgical resections, and a variety of bacterial or inflammatory diseases affecting the small intestine.[1] The amount of vitamin B_{12} absorbed is directly proportional to the length of functional intestine. Vitamin B_{12} deficiency due to insufficient dietary intake is rare and can occur only after years of abstinence from all animal products.

Elevated levels of vitamin B_{12} have been associated with pregnancy, the use of oral contraceptives and multivitamins, and in myeloproliferative diseases such as chronic granulocytic leukemia and myelomonocytic leukemia. An elevated vitamin B_{12} level in itself has not been known to cause clinical problems.

METHODOLOGY

The Access Vitamin B_{12} assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel along with alkaline potassium cyanide and dithiothreitol. This treatment denatures B_{12} binding proteins and converts all forms of vitamin B_{12} to the cyanocobalamin form. After neutralization, intrinsic factor-alkaline phosphatase conjugate and paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-intrinsic factor are added to the sample. Vitamin B_{12} in the sample binds to the intrinsic factor conjugate, preventing the conjugate from binding to the solid phase anti-intrinsic factor.

After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of vitamin B_{12} in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing and storing blood samples:[5]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 24 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Avoid assaying hemolyzed samples.

REAGENTS

PRODUCT INFORMATION

Access Vitamin B_{12} Reagent Pack

Cat. No. 33000: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 14 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-intrinsic factor complexes, TRIS buffered saline, surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	Borate buffer with surfactant, cobinamide, and < 0.1% sodium azide.
R1c:	Porcine intrinsic factor-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, surfactant, human serum albumin (HSA), < 0.1% sodium azide, and 0.25% ProClin 300.
R1d:	0.5N sodium hydroxide solution (NaOH) with 0.005% potassium cyanide (KCN), CORROSIVE.
R1e:	0.02% acetic acid solution with dithiothreitol (DTT).

*ProClin™ is a trademark of The Dow Chemical Company (“Dow”) or an affiliated company of Dow.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis C (HCV) and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[6]
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

> **⚠ CAUTION**
>
> **Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).**
> **To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.**

GHS HAZARD CLASSIFICATION

Particles (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Neutralizing Solution (Compartment R1b)	DANGER	
		
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
		Sodium Borate Decahydrate 1 - 3%
Conjugate (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.

	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Denaturing Reagent (Compartment R1d)	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Sodium Hydroxide 1 - 3%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Vitamin B_{12} Calibrators
   Provided at zero and approximately 100, 250, 500, 900, and 1,500 pg/mL (74, 184, 369, 664, and 1,107 pmol/L)
   Cat. No. 33005
2. Quality Control (QC) materials: commercial control material.
3. Access Sample Diluent A
   Vial Cat. No. 81908
   Diluent Pack Cat. No. A79783 (For use with the UniCel DxI system onboard dilution feature.)
4. Access Substrate
   Cat. No. 81906
5. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793
6. Access Vitamin B_{12} Calibrator S0
   Cat. No. 33006

EQUIPMENT AND MATERIALS

R1	Access Vitamin B_{12} Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

An active calibration curve is required for all tests. For the Access Vitamin B_{12} assay, calibration is required every 21 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a “random access” format rather than a “batch” format, quality control materials should be included in each 24-hour time period.[7] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use forty-five (45) µL of sample for each determination in addition to the sample container and system dead volumes. Use sixty-six (66) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is pg/mL. To change sample reporting units to the International System of Units (SI units), pmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply pg/mL by multiplication factor 0.7378.

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS INTERPRETATION

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

EXPECTED RESULTS

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Sera from 106 normal subjects and 60 diagnosed vitamin B_{12} deficient patients were assayed to establish expected ranges. The diagnosis of vitamin B_{12} deficiency was based on mean corpuscular volume, hematocrit, the presence of megaloblastic cells in bone marrow aspirates, and by vitamin B_{12} RIA. A non-parametric estimate at the 95% confidence level yields the following ranges:

Units	Normal Range	Indeterminate Range	Deficient Range
pg/mL	180-914	145-180	≤ 145
pmol/L	133-675	107-133	≤ 107

3. Evaluation of vitamin B_{12} deficiency should not depend on results from a single test. Complete evaluation should include other deficiency function tests and results from a physician's clinical evaluation.

PROCEDURAL NOTES

LIMITATIONS

1. Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value (approximately 50-1,500 pg/mL [37-1,107 pmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 50 pg/mL [< 37 pmol/L]). When the DxI system onboard dilution feature is used, the system will report results as less than 1,275 pg/mL (941 pmol/L).
   - If a sample contains more than the stated value of the highest Access Vitamin B_{12} Calibrator (S5), report the result as greater than that value (i.e., > 1,500 pg/mL [> 1,107 pmol/L]). Alternatively, dilute one volume of sample with 4 volumes of Access Vitamin B_{12} Calibrator S0 (zero) which is also available as Access Vitamin B_{12} Calibrator S0, Cat. No. 33006 or dilute one volume of sample with 4 volumes of Access Sample Diluent A, Cat No. 81908. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request.The system reports the results adjusted for the dilution.

     The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 4 volumes of Access Sample Diluent A, allowing samples to be quantitated up to approximately 7,500 pg/mL (5,533 pmol/L). The system reports the results adjusted for the dilution.
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[8,9]

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. Approximately 50% of patients with pernicious anemia have intrinsic factor antibodies.[10] The initial denaturation step in the Access Vitamin B_{12} assay inactivates intrinsic factor blocking antibodies. However, in very rare cases, some samples may not be inactivated due to the heterogeneity or extremely high titer of the intrinsic factor antibodies.[11] Such interfering antibodies may cause erroneous results. Patients should be further evaluated if suspected of having these antibodies or if the Vitamin B_{12} results are in conflict with other clinical or laboratory findings.

4. The Access Vitamin B_{12} results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.
5. This assay is not validated for testing neonatal or myeloproliferative syndrome specimens for vitamin B_{12} levels.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

METHODS COMPARISON

A comparison of 161 values using the Access Vitamin B_{12} assay on the Access Immunoassay System and a commercially available immunoassay kit gave the following statistical data:

n	Range of Observations (pg/mL)	Intercept (pg/mL)	Slope	Correlation Coefficient (r)
161	75-1,446	-20.0	0.95	0.97

A comparison of 46 values obtained by assaying clinical samples of serum and plasma (heparin) using the Access Vitamin B_{12} assay kit gave the following statistical data:

n	Range of Observations (pg/mL)	Intercept (pg/mL)	Slope	Correlation Coefficient (r)
46	141.37-543.30	46.4	0.86	0.937

DILUTION RECOVERY (LINEARITY)

Volumetric dilution of three samples containing various levels of Access Vitamin B_{12} Calibrator S0 (zero) gave the following data:

Sample 1	Expected Concentration (pg/mL)	Determined Concentration (pg/mL)	Recovery (%)
Neat	N/A	1,405	N/A
1/2	703	744	106
1/3	468	549	117
1/5	281	307	109
1/10	141	137	97
1/15	94	108	115
		Mean % Recovery	**109**

Sample 2	Expected Concentration (pg/mL)	Determined Concentration (pg/mL)	Recovery (%)
Neat	N/A	1,451	N/A
1/2	726	799	110
1/3	484	523	108
1/5	290	294	101
1/10	145	148	102
1/15	97	105	108
		Mean % Recovery	**106**

Sample 3	Expected Concentration (pg/mL)	Determined Concentration (pg/mL)	Recovery (%)
Neat	N/A	989	N/A
1/2	495	476	96
1/3	330	348	105
1/5	198	208	105
1/10	99	119	120
1/15	66	74	112
		Mean % Recovery	**108**

SPIKING RECOVERY

Addition of four different levels of cyanocobalamin to two patient samples with low vitamin B_{12} concentrations resulted in the following data:

Sample 1 (pg/mL)	Expected Concentration (pg/mL)	Determined Concentration (pg/mL)	Recovery (%)
Neat	N/A	91	N/A
100	191	194	102
400	491	520	106
800	891	966	108
1,200	1,291	1,323	102
		Mean % Recovery	**105**

Sample 2 (pg/mL)	Expected Concentration (pg/mL)	Determined Concentration (pg/mL)	Recovery (%)
Neat	N/A	105	N/A
100	205	180	88
400	505	494	98
800	905	923	102
1,200	1,305	1,373	105
		Mean % Recovery	**98**

IMPRECISION

This assay exhibits total imprecision of < 12% across the assay range. One study, using commercially available human serum based control material, performed by running two replicates of each sample per assay in two assays per day, provided the following data. The data were analyzed via analysis of variance (ANOVA):[12,13]

Sample	n	Grand Mean (pg/mL)	Within Run (%CV)	Total Imprecision (%CV)
1	20	88	5.0	8.5
2	40	374	4.8	6.6
3	40	775	6.9	7.5
4	40	975	11.4	11.4

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, 9 g/dL (90 g/L) total protein, and lipemic samples containing the equivalent of 1,800 mg/dL (20.32 mmol/L) triglycerides do not affect the concentration of vitamin B_{12} assayed.

Serum samples spiked with 10,000 pg/mL (7,378 pmol/L) of the vitamin B_{12} analog cobinamide exhibit < 0.5% cross-reactivity.

ANALYTICAL SENSITIVITY

The lowest detectable level of vitamin B_{12} distinguishable from zero (Access Vitamin B_{12} Calibrator S0) with 95% confidence is 50 pg/mL (37 pmol/L). This value is determined by processing a complete six point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision L

IFU updated to add Dutch, Finnish, Macedonian, Traditional Chinese, and Estonian

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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