ARCHITECT

SYSTEM

en

B12

REF 7K61

49-3244/R6

B7K610

Read Highlighted Changes
Revised August, 2010

B12

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

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.

Key to symbols used

REF	List Number	CONTROL L	Control Low, Medium, High (L, M, H)
IVD	*In Vitro* Diagnostic Medical Device	ASSAY CD-ROM	Assay CD-ROM
LOT	Lot Number	REACTION VESSELS	Reaction Vessels
	Expiration Date	SAMPLE CUPS	Sample Cups
2°C 8°C	Store at 2-8°C	REPLACEMENT CAPS	Replacement Caps
		REAGENT LOT	Reagent Lot
	Consult instructions for use	WARNING: REPRODUCTION HAZARD	Warning: Reproduction Hazard
CAL A	Calibrator A-F	WARNING: SENSITIZER	Warning: May cause an allergic reaction
SN	Serial Number	CONTAINS: AZIDE	Contains sodium azide. Contact with acids liberates very toxic gas.
SEPTUM	Septum	WARNING: SEVERE IRRITANT	Warning: Severe Irritant
	Manufacturer		

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

NAME

ARCHITECT B12

INTENDED USE

The ARCHITECT B12 assay is a Chemiluminescent Microparticle Intrinsic Factor assay for the quantitative determination of vitamin B12 in human serum and plasma on the ARCHITECT *i* System.

SUMMARY AND EXPLANATION OF TEST

Vitamin B12 (B12), a member of the corrin family, is a cofactor for the conversion of methylmalonyl Coenzyme-A (CoA) to succinoyl CoA. In addition, B12 is a cofactor in the synthesis of methionine from homocysteine, is implicated in the formation of myelin, and, along with folate, is required for DNA synthesis.[1,2]

B12 is absorbed from food after binding to a protein called intrinsic factor which is produced by the stomach. Causes of vitamin B12 deficiency can be divided into three classes: nutritional deficiency, malabsorption syndromes, and other gastrointestinal causes. B12 deficiency can cause megaloblastic anemia (MA), nerve damage and degeneration of the spinal cord. Lack of B12, even mild deficiencies, damages the myelin sheath that surrounds and protects nerves, which may lead to peripheral neuropathy. The nerve damage caused by a lack of B12 may become permanently debilitating, if the underlying condition is not treated. People with intrinsic factor defects who do not get treatment eventually develop a MA called pernicious anemia (PA).[2]

The relationship between B12 levels and MA is not always clear in that some patients with MA will have normal B12 levels; conversely, many individuals with B12 deficiency are not afflicted with MA. Despite these complications, however, in the presence of MA (e.g., elevated mean corpuscular volume (MCV)) there is usually serum B12 or folate deficiency.[2,3]

The true prevalence of B12 deficiency in the general population is unknown but increases with age. In one study,[4] fifteen percent of adults older than 65 years old had laboratory evidence of vitamin B12 deficiency.

A serum B12 level below the normal expected range may indicate that tissue B12 levels are becoming depleted. However, a B12 level in the low normal range does not ensure that B12 levels are healthy and symptomatic patients should be further evaluated with tests for holotranscobalamin,[5] homocysteine and methylmalonic acid.[6,7]

There are a number of conditions that are associated with low serum B12 levels, including iron deficiency, normal near-term pregnancy, vegetarianism, partial gastrectomy/ileal damage, celiac disease, use of oral contraception, parasitic competition, pancreatic deficiency, treated epilepsy, and advancing age.[2,8-10,11] Disorders associated with elevated serum B12 levels include renal failure, liver disease, and myeloproliferative diseases.[8,12]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT B12 assay is a two-step assay with an automated sample pretreatment, for determining the presence of B12 in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex.

Sample and Pre-Treatment Reagent 1, Pre-Treatment Reagent 2, and Pre Treatment Reagent 3 are combined. An aliquot of the pre-treated sample is aspirated and transferred into a new Reaction Vessel (RV). The pre-treated sample, assay diluent, and intrinsic factor coated paramagnetic microparticles are combined. B12 present in the sample binds to the intrinsic factor coated microparticles. After washing, B12 acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of B12 in the sample and the RLUs detected by the ARCHITECT *i* optical system.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT B12 Reagent Kit (7K61)

- **MICROPARTICLES** 1 Bottle (6.6 mL per 100 test bottle / 27.0 mL per 500 test bottle) Intrinsic Factor (porcine) coated Microparticles in borate buffer with protein (bovine) stabilizers. Minimum Concentration: 0.1% solids. Preservative: antimicrobial agents.
- **CONJUGATE** 1 Bottle (5.9 mL per 100 test bottle / 26.3 mL per 500 test bottle) B12 acridinium-labeled Conjugate in MES buffer. Minimum concentration: 0.7 ng/mL. Preservative: ProClin.
- **ASSAY DILUENT** 1 Bottle (10.0 mL per 100 test bottle / 51.0 mL per 500 test bottle) B12 Assay Diluent containing borate buffer with EDTA. Preservative: antimicrobial agents.
- **PRE-TREATMENT REAGENT 1** 1 Bottle (27.0 mL per 100 test bottle / 50.4 mL per 500 test bottle) B12 Pre-Treatment Reagent 1 containing 1.0 N sodium hydroxide with 0.005% potassium cyanide.
- **PRE-TREATMENT REAGENT 2** 1 Bottle (5.5 mL per 100 test bottle / 25.9 mL per 500 test bottle) B12 Pre-Treatment Reagent 2 containing alpha monothioglycerol and EDTA.
- **PRE-TREATMENT REAGENT 3** 1 Bottle (5.5 mL per 100 test bottle / 25.9 mL per 500 test bottle) B12 Pre-Treatment Reagent 3 containing cobinamide dicyanide in borate buffer with protein (avian) stabilizers. Preservative: sodium azide.

Assay Diluent

ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50)

- **MULTI-ASSAY MANUAL DILUENT** 1 Bottle (100 mL) ARCHITECT *i* Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- 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.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[13] Biosafety Level 2[14] or other appropriate biosafety practices[15,16] should be used for materials that contain or are suspected of containing infectious agents.

The following warnings and precautions apply to these components:

- Microparticles
- Assay Diluent
- Pre-Treatment Reagent 3

WARNING:	Contains sodium borate.
H361	Suspected of damaging fertility or the unborn child.
Prevention	
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P281	Use personal protective equipment as required.
Response	
P308+P313	If exposed or concerned: Get medical advice / attention.
Storage	
P405	Store locked up.

This material and its container must be disposed of in a safe way.

The following warnings and precautions apply to this component:

- Pre-Treatment Reagent 2

WARNING:	Contains monothioglycerol.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H303	May be harmful if swallowed.
H313	May be harmful in contact with skin.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P332+P313	If skin irritation occurs: Get medical advice / attention.
P362	Take off contaminated clothing and wash before reuse.
P312	Call a POISON CENTER or doctor / physician if you feel unwell.
This material and its container must be disposed of in a safe way.	

The following warnings and precautions apply to this component:

- Conjugate

WARNING:	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapours / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P363	Wash contaminated clothing before use.
This material and its container must be disposed of in a safe way.	

The following warnings and precautions apply to this component:

- Pre-Treatment Reagent 1

DANGER:	Contains sodium hydroxide.
H314	Causes severe skin burns and eye damage.
H290	May be corrosive to metals.
Prevention	
P234	Keep only in original container.
P260	Do not breathe mist / vapours / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P301+P330 +P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361 +P353	IF ON SKIN (or hair): Remove / Take off immediately all contaminated clothing. Rinse skin with water / shower.
P363	Wash contaminated clothing before reuse.
P310	Immediately call a POISON CENTER or doctor / physician.
P390	Absorb spillage to prevent material damage.
Storage	
P405	Store locked up.
P406	Store in corrosive resistant container with a resistant inner liner.
This material and its container must be disposed of in a safe way.	

- The Pre-Treatment Reagent 3 contains sodium azide. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a reagent kit or between reagent kits.**
- Before loading the ARCHITECT B12 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septa MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septa are not used according to the instructions in this package insert.**
  - Prolonged exposure of B12 Pre-Treatment Reagent 1 to air may compromise performance.
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

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The ARCHITECT B12 Reagent Kit must be stored at 2-8˚C in an upright position and may be used immediately after removal from 2-8˚C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT B12 Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septa and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT B12 assay file must be installed on the ARCHITECT *i* System from an ARCHITECT *i* System assay CD-ROM before performing the assay. For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT B12 assay is pg/mL. An alternate result unit, pmol/L, may be selected for reporting results by editing assay parameter "Result concentration units", to pmol/L. The conversion factor used by the system is 0.7378.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

The following specimen tube types were verified for use with the ARCHITECT B12 assay:

Glass	Plastic
Serum	Serum
	Serum separator
	Lithium heparin plasma separator
	Sodium heparin
	Dipotassium EDTA

The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT B12 assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - hemolyzed
  - obvious microbial contamination
- Performance has not been established for the use of body fluids other than human serum and plasma.
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at > 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter or
  - they were frozen and thawed.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- Transfer clarified specimen to a sample cup or secondary tube for testing.

Storage

- Specimens may be stored on or off the clot, red blood cells, or separator gel for
  - up to 3 days at room temperature or
  - up to 7 days at 2-8°C.
- If testing will be delayed more than 3 days for specimens stored at room temperature or more than 7 days for specimens stored at 2-8°C, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.
- Avoid more than three freeze/thaw cycles.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped frozen (dry ice) -20°C or colder. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 7K61 ARCHITECT B12 Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT *i* **ASSAY CD-ROM**
- 7K61-01 ARCHITECT B12 Calibrators
- 7K61-10 ARCHITECT B12 Controls
- 7D82-50 ARCHITECT *i* Multi-Assay Manual Diluent
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional).
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT B12 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
- Load the ARCHITECT B12 Reagent Kit on the ARCHITECT *i* System. Verify that all necessary assay reagents are present. Ensure that septa are present on all reagent bottles. Refer to ARCHITECT Operations Manual, Section 5, for details on how to load reagents.

- Order calibration, if necessary.
- For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
- For information on ordering patient specimens, calibrators and controls and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Order List Report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
  - Priority: 87 µL for the first B12 test plus 37 µL for each additional B12 test from the same sample cup
  - ≤ 3 hours onboard: 150 µL for the first B12 test plus 37 µL for each additional B12 test from the same sample cup
  - > 3 hours onboard: replace with a fresh sample (patient specimens, controls, and calibrators).
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
  - Prepare calibrators and controls.
  - Mix the ARCHITECT B12 Calibrators and Controls by gentle inversion before use.
  - To obtain the recommended volume requirements for the ARCHITECT B12 Calibrators and Controls, hold the bottles **vertically**, and dispense 3 drops of each calibrator or 3 drops of each control into each respective sample cup.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Specimens with a B12 value exceeding 2000 pg/mL (1476 pmol/L) are flagged with the code ">2000" when working in pg/mL (">1476" when working in pmol/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

- If using the Automated Dilution Protocol, the system performs a 1:3 dilution. The system will use the dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.
- Manual dilutions should be performed as follows:
  - The suggested dilution for B12 is 1:4.
  - For a 1:4 dilution, add 100 µL of the patient specimen to 300 µL of ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
  - The suggested dilution for specimens that generate repeated (2 or more) "3350 Unable to process test-aspiration error for (Sample Pipettor) at (RV 24)" errors is 1:2.
  - For a 1:2 dilution, add 100 µL of the patient specimen to 100 µL of ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
  - The operator must enter the dilution factor in the patient or control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The result before the dilution factor is applied must be greater than 83 pg/mL (61 pmol/L).

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT B12 calibration, test Calibrators A through F in duplicate. Calibrators should be priority loaded.
- Calibration Range: 0-2000 pg/mL (0-1476 pmol/L).
- A single sample of each control level must be tested to evaluate the assay calibration.
  - Order controls as described above.
  - Ensure that assay control values are within the concentration ranges specified in the control package insert.
- Once an ARCHITECT B12 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT B12 assay is a single sample of all control levels tested once every 24 hours each day of use. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Control values must be within the ranges specified in the control package insert. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected. Adversely affected test results are invalid, and these samples must be retested. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT B12 assay belongs to method group 1.

RESULTS

The ARCHITECT B12 assay utilizes a 4 Parameter Logistic Curve Fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

Alternate Result Units

- The default result unit for the ARCHITECT B12 assay is pg/mL. When the alternate result unit, pmol/L, is selected, the conversion factor used by the system is 0.7378.
- Conversion Formula:
  (Concentration in pg/mL) x (0.7378) = pmol/L
  (Concentration in pmol/L) / (0.7378) = pg/mL

Flags

- Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- The diagnosis of B12 deficiency cannot be solely based on serum or plasma B12 levels. Further testing for folic acid, intrinsic factor blocking antibodies, holotranscobalamin,[5] homocysteine, and/or methylmalonic acid is suggested for symptomatic patients with hematological or neurological abnormalities.[6,7]
- If the B12 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Hemolysis has been demonstrated to exhibit negative interference in this B12 assay. Hemolyzed specimens should not be analyzed.
- Specimens containing above normal protein concentrations may generate repeated (2 or more) "3350 Unable to process test-aspiration error for (Sample Pipettor) at (RV 24)" errors and should be quantified using the Automated Dilution Protocol or Manual Dilution Procedure (1:2).
- Heterophilic antibodies and rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[17] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- The assay is designed to test human serum and plasma. Specimens tested in other matrices may not give accurate results.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

EXPECTED VALUES

B12 Normals

It is recommended that each laboratory establish its own range, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A2.[18] Serum specimens from 143 individuals with normal mean corpuscular volume, homocysteine, and folate results were assayed for B12 using the ARCHITECT B12 assay. The B12 concentration range for this population was 141 to > 1218 pg/mL (104 to > 899 pmol/L) with a mean of 407 pg/mL (300 pmol/L). The central 95% of the sample population is defined below:

Expected Range	187-883 pg/mL	(138-652 pmol/L)

B12 Indeterminates

Levels above 300 or 400 pg/mL (221 or 295 pmol/L) are rarely associated with B12 deficiency induced hematological or neurological disease, respectively. Further testing is suggested for symptomatic patients with B12 levels between 100 and 300 pg/mL (74 and 221 pmol/L) (hematological abnormalities), and between 100 and 400 pg/mL (74 and 295 pmol/L) (neurological abnormalities).[6,7]

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from the data presented in the following studies.

Precision

The ARCHITECT B12 assay is designed to have a Total CV of ≤ 11% for concentrations in the range of the low, medium, and high controls. A 20-day precision study was performed for the ARCHITECT B12 assay based on guidance from the CLSI document EP5-A2.[19] Testing was conducted at Abbott Laboratories using three ARCHITECT B12 assay reagent lots, two calibrator lots, one control lot, and two instruments. Four levels of controls and panels were assayed in replicates of three at two separate times of day for 20 different days. The data are summarized in the following table.

Instrument	Sample	n	Mean (pg/mL)	Within-Run SD	Within-Run %CV	Within Laboratory Precision (Total) SD	Within Laboratory Precision (Total) %CV
1	Serum Panel	360	262	12.6	4.8	16.3	6.2
	Low Control	354	246	13.8	5.6	16.7	6.8
	Medium Control	355	424	14.3	3.4	16.8	4.0
	High Control	359	890	36.0	4.0	38.9	4.4
2	Serum Panel	357	248	11.6	4.7	13.3	5.4
	Low Control	356	241	10.4	4.3	12.9	5.4
	Medium Control	352	408	13.3	3.3	15.5	3.8
	High Control	355	885	23.9	2.7	29.7	3.4

Accuracy by WHO

A study was conducted to evaluate the accuracy of the ARCHITECT B12 assay using the B12 World Health Organization International Standard 03/178. The assay demonstrated a -3.6% difference from the target value of 480 pg/mL (354 pmol/L).

Sensitivity

Sensitivity is defined as the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the ARCHITECT B12 assay. The assay is designed to have an LoQ of ≤ 150 pg/mL (≤ 111 pmol/L). A study conducted based on guidance from CLSI document EP17-A[20] produced an LoB of 83 pg/mL (61 pmol/L), an LoD of 125 pg/mL (92 pmol/L) and LoQ of 125 pg/mL (92 pmol/L).

Specificity

The ARCHITECT B12 assay is designed to have an interference (difference) less than the LoD of the assay with cobinamide, a B12 analogue. The specificity of the ARCHITECT B12 assay was determined by studying the cross reactivity with cobinamide. A human serum specimen at approximately 230 pg/mL (168 pmol/L) was supplemented with cobinamide at 9000 pg/mL and the resulting interference was 4 pg/mL (3 pmol/L).

Interference

At the concentrations listed below, bilirubin (conjugated and unconjugated), total protein, and triglycerides showed less than 10% interference in the ARCHITECT B12 assay for low samples (concentration range: 150 pg/mL to 250 pg/mL (111 pmol/L to 184 pmol/L)) and higher samples (concentration range: > 500 pg/mL (> 369 pmol/L)):

- Bilirubin < 25.1 mg/dL
- Total Protein < 12 g/dL
- Triglycerides < 3325 mg/dL

Hemolyzed specimens should not be analyzed; refer to the **LIMITATIONS OF THE PROCEDURE** section of this package insert.

Accuracy by Correlation

A study was conducted based on guidance from CLSI document EP9-A2.[21] Three hundred and twenty nine serum specimens were tested for the determination of B12 using the ARCHITECT B12 assay and a commercially available diagnostic kit. The specimen testings are shown in the following table*.

Abbott ARCHITECT B12 vs AxSYM B12

Method	Number of Specimens	Intercept	Slope	Correlation Coefficient
Least Squares Linear Regression	329	-2.05	1.01	0.99
Passing-Bablok Linear Regression[22]	329	21.96	0.95	0.99

In this evaluation, serum specimens tested ranged from 113 to 2769 pg/mL (83 to 2043 pmol/L) by the ARCHITECT B12 assay, and from 93.5 to 2655.8 pg/mL (69.1 to 1959.5 pmol/L) by the comparator assay.

* Representative data, results in individual labs may vary from these data.

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Abbott Ireland
Diagnostics Division
Lisnamuck, Longford
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August 2010