Alinity i B12-07

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i B12 assay is a chemiluminescent microparticle Intrinsic Factor assay used for the quantitative determination of vitamin B12 in human serum and plasma on the Alinity i analyzer.

SUMMARY AND EXPLANATION OF THE 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 adequate 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*, *9*, *10*, *11* Disorders associated with elevated serum B12 levels include renal failure, liver disease, and myeloproliferative diseases.*8*, *12*

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the quantitative determination of vitamin B12 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, Pre-Treatment Reagent 1, Pre-Treatment Reagent 2, and Pre-Treatment Reagent 3 are combined. An aliquot of the pretreated sample, intrinsic factor coated paramagnetic microparticles, and assay diluent are combined and incubated. The B12 present in the sample binds to the intrinsic factor coated microparticles. The mixture is washed. B12 acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of B12 in the sample and the RLUs detected by the system optics.

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity i B12 Reagent Kit 07P67

NOTE: This product is composed of 6 components, which are packaged as a 2 cartridge reagent set. Both cartridges are required to perform the assay.

Volumes (mL) listed in the table below indicate the volume per cartridge set.

REF	07P6721	07P6731
Tests per cartridge set	100	600
Number of cartridge sets per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	5.4 mL	24.8 mL
CONJUGATE	4.9 mL	24.3 mL
ASSAY DILUENT	8.1 mL	42.8 mL
PRE-TREATMENT REAGENT 1	48.1 mL	48.1 mL
PRE-TREATMENT REAGENT 2	5.3 mL	24.6 mL
PRE-TREATMENT REAGENT 3	5.3 mL	24.8 mL

MICROPARTICLES Intrinsic Factor (porcine) coated microparticles in borate buffer with protein (bovine) stabilizers. Minimum concentration: 0.1% solids. Preservative: antimicrobial agents.

CONJUGATE B12 acridinium-labeled conjugate in MES buffer. Minimum concentration: 0.7 ng/mL. Preservative: ProClin 300.

ASSAY DILUENT Borate buffer with EDTA. Preservative: antimicrobial agents.

PRE-TREATMENT REAGENT 1 1.0 N sodium hydroxide with 0.005% potassium cyanide.

PRE-TREATMENT REAGENT 2 Alpha monothioglycerol and EDTA.

PRE-TREATMENT REAGENT 3 Cobinamide dicyanide in borate buffer with protein (avian) stabilizers. Preservative: sodium azide.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

- Rx ONLY

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. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[13], [14], [15], [16]

The following warnings and precautions apply to: MICROPARTICLES/ASSAY DILUENT	
	
DANGER	Contains disodium tetraborate decahydrate.
H360	May damage fertility or the unborn child.
Prevention	
P201	Obtain special instructions before use.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P308+P313	IF exposed or concerned: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: PRE-TREATMENT REAGENT 3	
	
DANGER	Contains disodium tetraborate decahydrate and sodium azide.
H360	May damage fertility or the unborn child.
EUH032	Contact with acids liberates very toxic gas.
Prevention	

P201	Obtain special instructions before use.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P308+P313	IF exposed or concerned: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: PRE-TREATMENT REAGENT 2

WARNING	Contains monothioglycerol.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
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 water.
P332+P313	If skin irritation occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

The following warnings and precautions apply to: 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 / vapors / 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.
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): Take off immediately all contaminated clothing. Rinse skin with water / shower.
P310	Immediately call a POISON CENTER or doctor / physician.
P390	Absorb spillage to prevent material damage.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: CONJUGATE

WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.

Prevention	
P261	Avoid breathing mist / vapors / 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 water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

- Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 48 hours before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 48 hours before use.
Onboard	System Temperature	26 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i B12 assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
pg/mL	0.7378	pmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Specimen Types	Collection Tubes
Serum	Serum
Plasma	Lithium heparin plasma separator

Specimen Conditions

Do not use:

- heat-inactivated specimens

- pooled specimens
- hemolyzed specimens
- specimens with obvious microbial contamination

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum and plasma specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at a minimum of 100 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

  Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Minimum Centrifugation time (minutes)} = \frac{100\,000 \text{ g-minutes}}{\text{RCF}}$$

Recentrifugation Time (Minutes)	RCF (x g)*	g-Minutes
10	10 000	100 000
20	5000	100 000
40	2500	100 000

* To ensure consistency in results, specimens must be centrifuged using an appropriate tube at a minimum of 2500 RCF to obtain a minimum of 100 000 g-minutes.

$RCF = 1.12 \times r_{max} (rpm/1000)^2$

RCF -	The relative centrifugal force generated during centrifugation.
rpm -	The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).
Centrifugation Time -	The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.
r_{max} -	Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (r_{max}) should be manually measured in millimeters and the RCF calculated.
g-minutes -	The unit of measure for the product of RCF (× g) and centrifugation time (minutes).

· Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature	3 days	Specimens may be stored in the primary or aliquot tube.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
	2 to 8°C	7 days	Specimens may be stored in the primary or aliquot tube.

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 primary tube and store at -20°C or colder.

Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

PROCEDURE

Materials Provided

07P67 Alinity i B12 Reagent Kit

Materials Required but not Provided

- Alinity i B12 assay file
- 07P6702 Alinity i B12 Calibrators
- 07P6712 Alinity i B12 Controls or other commercially available controls
- Alinity Pre-Trigger Solution
- Alinity Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required **for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 87 µL
- Sample volume for each additional test from same sample cup: 37 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 37 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i B12 calibrator package insert and/or Alinity i B12 control package insert for preparation and usage.
- For general operating procedures, **refer to the Alinity ci-series Operations Manual, Section 5.**
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples cannot be diluted for the Alinity i B12 assay.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a 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.

Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

- If statistically-based quality control limits are not available, then the calibration should not exceed a 30-day limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i B12 assay is that a single sample of each control level be tested once every day testing performed.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished **by assaying a minimum of 20 replicates over several (3-5) days** and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.*17*

- If quality control results do not meet the acceptance criteria defined by laboratory QC procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. **For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.*18*

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i B12 assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

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 Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in pg/mL (pmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity i B12 assay is **148 to 2000 pg/mL** (109 to 1476 pmol/L).

LIMITATIONS OF THE PROCEDURE

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- 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 recommended 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) sample aspiration errors. These specimens are unable to be tested using the Alinity i B12 assay.**
- Heterophilic antibodies and rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. 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.[19]

- The assay is designed to test human serum and lithium heparin 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

This study was performed on the ARCHITECT i System.

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Manufacurer provided refrence ranges will be adopted, effort made to verify locally

B12 Normals

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A2.*20* Serum specimens from 121 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 < 146 to 1218 pg/mL with a median of 409 pg/mL. The central 95% of the sample population (expected range) is defined below:

Expected Range	**213-816 pg/mL**	(157-602 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

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.*21* Testing was conducted

using 1 lot of the Alinity i B12 Reagent Kit, 1 lot of the Alinity i B12 Calibrators, and 1 lot of the Alinity i B12 Controls and 1 instrument. Three controls and 1 human serum panel were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

Sample	n	Mean (pg/mL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Low Control	119	251	10.4	4.2	13.5	5.4
Medium Control	120	451	17.9	4.0	21.9	4.9
High Control	120	929	28.7	3.1	30.6	3.3
Serum Panel 1	118	187	12.9	6.9	14.7	7.9

[a]Includes within-run, between-run, and between-day variability.

Sample	n	Mean (pmol/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Low Control	119	185	7.7	4.2	10.0	5.4
Medium Control	120	333	13.2	4.0	16.2	4.9
High Control	120	685	21.1	3.1	22.6	3.3
Serum Panel 1	118	138	9.5	6.9	10.8	7.9

[a]Includes within-run, between-run, and between-day variability.

Accuracy by WHO

This study was performed on the ARCHITECT i System.

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).**

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.*22* Testing was conducted using 4 lots of the Alinity i B12 Reagent Kit on each of 2 instruments over a minimum of 3

days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

	pg/mL	pmol/L
LoB[a]	83	61
LoD[b]	109	80
LoQ[c]	**148**	109

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of 10 %CV and a maximum allowable bias of 10% were met.

Linearity

A study was performed based on guidance from CLSI EP06-A.*23*

This assay is linear across the measuring interval of **148 to 2000** pg/mL (109 to 1476 pmol/L).

Analytical Specificity

This study was performed on the ARCHITECT i System.

The specificity of the ARCHITECT B12 assay was determined by studying the cross reactivity with cobinamide, a B12 analogue. A human serum specimen at 228 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

This study was performed on the ARCHITECT i System.

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07-A2.*24*

Potential interference in the ARCHITECT B12 assay from bilirubin (conjugated and unconjugated), total protein, and triglycerides was demonstrated in a study. The endogenous substances listed below were spiked into samples with different levels of B12 (150-250 pg/mL and > 500 pg/mL). The samples were assayed, and the B12 concentrations of the spiked samples were compared to the reference samples and showed less than 10% interference at the following test concentrations:

Potentially Interfering Substance	Concentration
Bilirubin	≤ 20 mg/dL
Total Protein	≤ 12 g/dL
Triglycerides	≤ 3000 mg/dL

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

Tube Type Matrix Comparison

This study was performed on the ARCHITECT i System.

A study was performed based on guidance from CLSI EP07-A2*24* to evaluate the types of blood collection tubes that can be used with the ARCHITECT B12 assay. The tube types were evaluated using the Passing-Bablok regression method to compare each evaluation tube type to the control tube type (serum plastic). One specimen set tested less than 200 pg/mL (Control = 173 pg/mL). The lithium heparin plasma separator tube for this specimen displayed a difference of 31 pg/mL (lithium heparin plasma separator tube = 204 pg/mL). The data are summarized in the following table.

Evaluation Tube Type	N	Control Tube (serum plastic) Range[a] (pg/mL)	Evaluation Tube Range[a] (pg/mL)	r[b]	Intercept (pg/mL)	Slope
Serum, glass	52	173-1102	174-1252	0.995	-8.47	1.02
Serum separator, plastic	52		175-1177	0.996	-1.30	1.02
Lithium heparin plasma separator	63	173-1866	204-1970	0.998	10.58	1.02

[a] Range of Specimen Means

[b] Correlation Coefficient

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.*25*

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity i B12	Serum	pg/mL	126	0.99	-12.84	1.04	171-1868
vs ARCHITECT B12	Serum	pmol/L	126	0.99	-8.92	1.04	126-1378

Carryover

No significant carryover (≤ 66 pg/mL) was observed when a sample at approximately 9 000 pg/mL to a low sample (below LOQ) was assayed.

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