



AXIS-SHIELD

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AxSYM[®] Active-B12

IVD **REF** 1P43-20

ABOL039/R1

CE

AxSYM[®] Active-B12 (Holotranscobalamin)


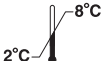




Customer Service

United States: 1-877-4ABBOTT

International: Call your Abbott Representative

This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

January 2007

Key to symbols used			
REF	List Number		Expiration Date
IVD	<i>In Vitro</i> Diagnostic Medical Device	LOT	Lot Number
	Store at 2-8°C	STANDARD CAL A	Standard Calibrator (A-F)
	Store at 15-30°C	CONTROL L	Control Low, High (L, H)
	CAUTION: Consult accompanying documents	REAGENT PACK	Reagent Pack
	Manufacturer	SAMPLE CUPS	Sample Cups
	Consult Instructions for Use	MATRIX CELLS	Matrix Cells
		REACTION VESSELS	Reaction Vessels

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

NAME

Active-B12

INTENDED USE

AxSYM® Active-B12 is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of holotranscobalamin (HoloTC) in human serum and plasma on the AxSYM System. HoloTC (vitamin B₁₂ bound to transcobalamin) is used as an aid in the diagnosis and treatment of vitamin B₁₂ deficiency.

SUMMARY AND EXPLANATION OF THE TEST

Vitamin B₁₂ (cobalamin) in serum is bound to two proteins, transcobalamin (TC) and haptocorrin (HC). The transcobalamin-vitamin B₁₂ complex is called holotranscobalamin (HoloTC). HoloTC contains the biologically available cobalamin because only HoloTC promotes the uptake of its cobalamin by all cells, via specific receptors. The much larger fraction (about 80 %) of cobalamin carried by HC is considered metabolically inert because no cellular receptors exist, except on the liver.¹

Genetic absence of HC, although rare, is not a serious condition.² On the other hand, genetic absence or abnormalities of TC manifest as typical hematological, neurological, and metabolic pathologies of cobalamin deficiency requiring aggressive treatment even though serum analysis can show normal cobalamin concentrations.^{3,4,5}

The shorter circulating half-life for HoloTC compared to HoloHC makes a decrease of HoloTC one of the earliest markers of cobalamin deficiency.^{6,7}

The measurement of total serum cobalamin suffers from some limitations, in particular, most of the cobalamin measured is that bound to HC.^{8,9,10,11} A number of studies have been published to support that HoloTC would be a better indicator of vitamin B₁₂ status than total serum cobalamin.^{12,13,14} New methods based on specific anti-TC antibodies have been available and confirm the usefulness of HoloTC for diagnosing B₁₂ deficiency.^{15,16} As expected, HoloTC levels are low in patients with biochemical signs of vitamin B₁₂ deficiency.¹⁷ Notably, low values have been reported in vegetarians^{18,19} vegans²⁰, and in populations with a low intake of vitamin B₁₂.^{21,22} Interestingly, a low serum HoloTC (but not vitamin B₁₂) was reported in patients with Alzheimer's disease compared to a healthy control group.²³ HoloTC levels reflect vitamin B₁₂ status, independent of recent absorption of the vitamin.²⁴

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM Active-B12 is based on Microparticle Enzyme Immunoassay (MEIA) technology. The AxSYM Active-B12 reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM Active-B12 reagents required for one test are pipetted by the Sampling Probe into various wells of a Reaction Vessel (RV).
- A reaction mixture is formed by combining diluted sample and microparticles coated with Anti-HoloTC monoclonal antibody in the sample well of the RV.
- When human HoloTC antigen is present in the sample, it binds to the coated microparticles, forming antigen-antibody complexes on the microparticles.
- The Anti-TC Antibody: Alkaline Phosphatase Conjugate is pipetted into a second well of the RV.
- The Active-B12 Wash Buffer is pipetted into a third well of the RV.
- The Matrix Cell Wash is pipetted into a fourth well of the RV.

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

PROCESSING CENTER

- An aliquot of the reaction mixture, containing microparticles and bound antigen-antibody complex, is transferred to the Matrix Cell. The microparticles bind irreversibly to the glass fiber matrix.
- The Matrix Cell is washed to remove materials not bound to the microparticles.
- The Anti-TC Antibody:Alkaline Phosphatase Conjugate is dispensed onto the Matrix Cell and it binds with the antigen-antibody complexes.
- The Matrix Cell is washed to remove conjugate not bound to the microparticles.

- The substrate, 4-Methylumbelliferyl Phosphate, is added to the Matrix Cell. The alkaline phosphatase-labeled conjugate catalyzes the removal of a phosphate group from the substrate, yielding the fluorescent product, 4-Methylumbelliferone. This fluorescent product is measured by the MEIA optical assembly.

For further information regarding MEIA technology, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS

REAGENT PACK, 100 Tests

AxSYM Active-B12 Reagent Pack (1P43-20)*

- 1 Bottle (8.4 mL) Anti-HoloTC (Mouse, Monoclonal) Antibody Coated Microparticles in TRIS buffer with protein (Bovine) stabilizers. Minimum concentration: 0.025% solids (w/v). Preservative: Sodium Azide. (Reagent Bottle 1)
- 1 Bottle (13.3 mL) Anti-TC Antibody:Alkaline Phosphatase Conjugate in TRIS buffer with protein (Bovine) stabilizers. Minimum concentration 0.1 µg/mL. Preservative: Sodium Azide. (Reagent Bottle 2)
- 1 Bottle (14.2 mL) Wash Buffer containing detergent. Preservative: ProClin® 300. (Reagent Bottle 3)
- 1 Bottle (33.0 mL) Matrix Cell Wash. Preservatives: Sodium Azide and Antimicrobial Agents. (Reagent Bottle 4)

*1P43-66 includes an AxSYM Active-B12 Reagent Pack (100 tests), RV's (100 each) and Matrix Cells (100 each). 1P43-20 includes these items for international shipments.

CALIBRATORS

AxSYM Active-B12 Standard Calibrators (1P43-01)

6 Bottles (4.3 mL each) of AxSYM Active-B12 Standard Calibrators. Calibrator A is phosphate buffer with protein (Bovine) stabilizers. Calibrators B-F contain HoloTC in phosphate buffer with protein (Bovine) stabilizers to yield the concentrations (pmol/L) in the following table.

Standard Calibrator	HoloTC Concentration (pmol/L)
STANDARD CAL A	0
STANDARD CAL B	8
STANDARD CAL C	16
STANDARD CAL D	32
STANDARD CAL E	64
STANDARD CAL F	128

Preservative: Sodium Azide.

The AxSYM Active-B12 Standard Calibrators are traceable to internal reference standards which underwent a one-time value assignment to align with another commercially available assay.

CONTROLS

AxSYM Active-B12 Controls (1P43-10)

2 Bottles (8.3 mL each) of AxSYM Active-B12 Controls containing HoloTC in processed human serum/phosphate buffer to yield the concentrations (pmol/L) in the following table. Infection risk.

Bottle	HoloTC Concentration (pmol/L)	Range (pmol/L)
CONTROL L	21	9 - 33
CONTROL H	48	26 - 70

Preservative: Sodium Azide.

Human serum nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV or HCV RNA.

OTHER REAGENTS

AxSYM Probe Cleaning Solution (9A35-05)

PROBE CLEANING SOLUTION 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammonium Hydroxide (TEAH).

Solution 1 (MUP) (8A47-04)

SOLUTION 1 | MUP 4 Bottles (230 mL each) Solution 1 (MUP) containing 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: Sodium Azide.

Solution 3 (Matrix Cell Wash) (8A81-04)

SOLUTION 3 | MATRIX CELL WASH 4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M Sodium Chloride in TRIS buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

SOLUTION 4 LINE DILUENT 1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M Phosphate buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

SAFETY PRECAUTIONS

- CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, refer to **CONTENTS** section of this package insert. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV or HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens,²⁵ Biosafety Level 2²⁶ or other appropriate biosafety practices^{27,28} should be used for materials that contain or are suspected of containing infectious agents.
- The AxSYM Active-B12 Wash Buffer contains methylisothiazolones and is classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.



- R43 May cause sensitization by skin contact.
 S24 Avoid contact with skin.
 S35 This material and its container must be disposed of in a safe way.
 S37 Wear suitable gloves.
 S46 If swallowed, seek medical advice immediately and show this container or label.

- The AxSYM Active-B12 Matrix Cell Wash contains sodium azide and is classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.



- R22 Harmful if swallowed.
 R32 Contact with acids liberates very toxic gas.
 S35 This material and its container must be disposed of in a safe way.
 S36 Wear suitable protective clothing.
 S46 If swallowed, seek medical advice immediately and show this container or label.

For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

Refer to AxSYM System Operations Manual, Section 8, for the safe disposal of these materials.

HANDLING PRECAUTIONS

- Do not use reagent packs beyond the expiration date, or a maximum of 336 cumulative hours on board the AxSYM System.
- Do not mix reagents from different reagent packs.
- AxSYM Active-B12 reagents are susceptible to bubbles/foaming and require inspection and removal of bubbles before loading. If bubbles are present, refer to the AxSYM System Operations Manual, Section 9, for instructions on removing bubbles from reagent packs.
- Do not use Solution 1 (MUP) beyond the expiration date or a maximum of 14 days on board the AxSYM System. When loading new Solution 1 (MUP), it is important to immediately tighten the instrument cap for MUP to minimize exposure to air. Prolonged exposure of MUP to air may compromise performance.**

Refer to the AxSYM System Operations Manual, Sections 7 and 8, for a more detailed discussion of the safety and handling precautions during system operation.

STORAGE INSTRUCTIONS

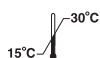


The AxSYM Active-B12 Reagent Pack, Standard Calibrator Pack and Control Pack must be stored at 2-8°C (do not freeze). They may be used immediately after removal from the refrigerator. Standard Calibrators and Controls should be returned to 2-8°C storage immediately after use.

When stored and handled as directed, reagents are stable until expiration date. The AxSYM Active-B12 Reagent Pack may be on board the AxSYM System for a maximum of 336 cumulative hours (for example, 42 eight hour shifts). Recalibration may be required to obtain maximum on board reagent stability. More frequent use of controls may be required to monitor reagent performance within the same lot. After 336 hours, the reagent pack must be discarded. Refer to the AxSYM System Operations Manual, Sections 2 and 5, and Appendix C, for further information on tracking on board time.



Solution 1 (MUP) must be stored at 2-8°C (do not freeze). It may be used immediately after removal from the refrigerator. It may be on board the AxSYM System for a maximum of 14 days. After 14 days it must be discarded.



The AxSYM Probe Cleaning Solution, Solution 3 (Matrix Cell Wash), and Solution 4 (Line Diluent) must be stored at 15-30°C.

INSTRUMENT PROCEDURE

Assay File Installation

The AxSYM Active-B12 Assay Disk Version 1.0 or higher must be installed on the AxSYM System from the software disk, 1P44-01 or higher, prior to performing the AxSYM Active-B12 assay. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

NOTE: AxSYM Active-B12 must only be used with AxSYM System Software version 5.0 or higher.

AxSYM Active-B12 Assay Parameters

The default assay parameters for the AxSYM Active-B12 assay are listed below. Assay parameters that can be edited contain a (>) symbol. These parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameter screen. Press PRINT to print the assay parameters.

Assay Parameters	
1	Long Assay Name (English): Active-B12
6	Abbrev Assay Name (English): ActiveB12
11	Assay Number: 880
12	Assay Version:*
13	Calibration Version:*
14	Assay File Revision: *
15	Assay Enabled > ON
17	Assay Type: MEIA
18	Standard Cal Reps > 2
21	Cal A Concentration: 0.00
22	Cal B Concentration: 8.00
23	Cal C Concentration: 16.00
24	Cal D Concentration: 32.00
25	Cal E Concentration: 64.00
26	Cal F Concentration: 128.00
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > pmol/L
46	Selected Result Decimal Places > 2

Refer to the AxSYM System Operations Manual for a detailed description of Instrument Procedures.

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma collected in lithium heparin plasma separator tubes may be used in the AxSYM Active-B12 assay. Other anticoagulants are not recommended for use with the AxSYM Active-B12 assay. Follow the manufacturer's processing instructions for specimen collection tubes.
- The AxSYM System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen type(s) is (are) used in the AxSYM Active-B12 assay.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter.
- If testing will be delayed, specimens should be separated from the clot or red blood cells. Specimens may be stored for up to 28 days at 2-8°C after the date of collection. If testing will be delayed more than 28 days, the specimen should be stored at -20°C or colder. Specimens stored frozen at -20°C or colder for 6 months did not show performance differences.
- Multiple freeze/thaw cycles should be avoided. Specimens may undergo up to 3 freeze/thaw cycles. Specimens must be mixed thoroughly after thawing, by LOW speed vortexing or by gently inverting, then centrifuged at $\geq 10,000 \times g$ for at least 5 minutes prior to testing to remove particulate matter and ensure consistency in the results.²⁹
- To minimize the effect of evaporation, all samples (patient specimens, calibrators and controls) should be tested within 3 hours of being placed on board the AxSYM System.
- Refer to the AxSYM System Operations Manual, Section 5, for a detailed discussion of on board sample storage constraints.
- Inspect samples for bubbles. Remove bubbles prior to analysis.
- Centrifuged specimens with a lipid layer on top of the liquid must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen and not the lipemic material.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances. Specimens may be shipped on wet or dry ice. Do not exceed storage temperature and time limitations as cited above. Prior to shipment, specimens must be removed from the clot or red blood cells.

SAMPLE VOLUME

The sample volume required to perform a single AxSYM Active-B12 test on the AxSYM System varies according to the type of sample container used. For sample cups, minimum sample volume required is 173 μL for STAT and ROUTINE. For every additional AxSYM Active-B12 test performed from the same sample container, an additional 123 μL sample is required.

The sample cup minimum volume for both STAT and ROUTINE tests is calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is (are) ordered and printed in the Orderlist Report. When using Host Order Query, the Order screen information and Orderlist Reports are not available.

Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query Option.

For sample volume requirements in Primary or Aliquot Tubes, and control volume requirements for multiple AxSYM Active-B12 reagent lots, refer to the AxSYM System Operations Manual, Section 5.

To obtain the recommended volume requirements for the AxSYM Active-B12 Standard Calibrator and Controls, hold the bottles vertically and dispense 7 drops of each Standard Calibrator or 7 drops of each Control into each respective sample cup.

AxSYM Active-B12 PROCEDURE

Materials Provided

- 1P43-66 (US)
 - 1P43-20 (international)
- AxSYM Active-B12 Reagent Pack, containing:
- AxSYM Active-B12 **REAGENT PACK**
 - 100 **REACTION VESSELS**
 - 100 **MATRIX CELLS**

Materials Required But Not Provided

- AxSYM System
- 1P43-01 AxSYM Active-B12 Standard Calibrators
- 1P43-10 AxSYM Active-B12 Controls
- 8A47-04 **SOLUTION 1 MUP**
- 8A81-04 **SOLUTION 3 MATRIX CELL WASH**
- 8A46 **SOLUTION 4 LINE DILUENT**
- 9A35-05 AxSYM **PROBE CLEANING SOLUTION**
- 8A76-01 **SAMPLE CUPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the Orderlist screen

CAUTION:

- AxSYM Active-B12 Standard Calibrators and Controls should be mixed by gentle inversion prior to use.
- When manually dispensing samples into sample cups, verify that dispensing equipment does not introduce cross-contamination and that it delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Assay Procedure

Sections 5 and 6 of the AxSYM System Operations Manual can be easily removed for use at the instrument. They contain detailed steps for performing assay calibration and sample testing procedures.

Prior to ordering tests, confirm that the System inventory of Matrix Cells, bulk solutions, and waste levels are acceptable.

The Orderlist Report contains sample placement information and sample cup requirements for all ordered tests. It is recommended that this report be referenced when loading samples into sample segments. When using Host Order Query, the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query Option.

CAUTION: When operating the AxSYM System, always observe the following:

- The System status must be WARMING, PAUSED, READY or STOPPED before adding or removing sample segments, reagent packs or RVs.
- Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in process. If opened, all processing will stop. All tests will be terminated and must be repeated.
- When testing is completed, it is recommended that the AxSYM Active-B12 Reagent Pack is removed from the Sampling Center to maximize the on board reagent pack use. Store at 2-8°C.

SPECIMEN DILUTION PROCEDURES

Manual Dilution Protocol

Patient specimens with HoloTC values exceeding 128.00 pmol/L (HIGH RANGE Assay Parameter 92) are flagged with the code ">128.00 pmol/L".

Patient specimens with HoloTC concentrations reported as greater than 128.00 pmol/L may be diluted using a manual dilution of 1:5. Add 100 μL the patient specimen to 400 μL of the AxSYM Active-B12 Low Control (1P43-10). Calculate the value of the patient specimen using the following equation:

$$\text{Specimen Value (pmol/L)} = 5 \times (\text{value of the diluted sample on AxSYM} - 17)$$

QUALITY CONTROL PROCEDURES

CALIBRATION

The AxSYM Active-B12 assay must be calibrated using the Active-B12 Standard Calibration (6-point) procedure.

Standard Calibration

To perform an AxSYM Active-B12 Standard Calibration, test the Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of both levels of Active-B12 controls must be tested as a means of evaluating the assay calibration.

Once the AxSYM Active-B12 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent pack with a new lot number is used.
- Controls are out of range.
- The MEIA Optics Verification Update has been performed.

Refer to the AxSYM System Operations Manual, Section 6, for:

- Setting up an assay calibration
- When recalibration may be necessary
- Calibration Verification

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendix E, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

QUALITY CONTROL

The minimum control requirement for an AxSYM Active-B12 assay is a single sample of both controls tested every 24 hours, each day of use for each reagent lot. Controls may be placed in any position in the Sample Carousel.

If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

Ensure that assay controls are within the concentration ranges specified. Refer to the REAGENTS, CONTROLS section of this package insert for AxSYM Active-B12 Control ranges.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When an Active-B12 control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and require retesting. Recalibration may be indicated. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5 for further information. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

RESULTS

UNITS

The results are printed with concentrations expressed in pmol/L.

CALCULATION

AxSYM Active-B12 uses a Four-Parameter Logistic Curve fit (4PLC, Y-weighted) to generate a calibration curve. Refer to the AxSYM System Operations Manual, Appendix F, for further information.

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 AxSYM System Operations Manual Sections 1 and 2.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, the AxSYM Active-B12 results should be used in conjunction with other clinical data; e.g., symptoms, medical history, etc.
- If the Active-B12 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).³⁰ Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.³¹ These specimens should not be assayed with the AxSYM Active-B12 assay.

- Heterophilic antibodies in human plasma can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. The presence of heterophilic antibodies in a patient may cause anomalous values to be observed.^{32,33}
- **AxSYM Active-B12 results should not be used interchangeably with other manufacturers' methods for HoloTC determinations.**
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert for additional information.

EXPECTED VALUES

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

In a representative study, serum specimens from 281 apparently healthy donors were tested using the AxSYM Active-B12 assay.³⁴ The mean HoloTC concentration (derived from log-transformed data to normalize the population) was 47.7 pmol/L with a range from 8.9 to 123.3 pmol/L. The central 95% of the population defined the expected range of 19.1 to 119.3 pmol/L.

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

The AxSYM Active-B12 assay is designed to have precision of $\leq 10\%$ total CV for concentrations in the range of the Active-B12 low and high panels. Precision was determined as described in the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2.³⁵ Human serum based panels were assayed in replicates of two at two separate times of the day for 20 days ($n = 80$ for each panel member). Testing was performed on two AxSYM Systems, using one reagent lot.

Data from this study are summarized in the following table:*

LOW PANEL						
Instrument	n	Mean Conc. Value (pmol/L)	Within Run		Total	
			SD	CV(%)	SD	CV(%)
1	80	23.3	1.03	4.4	1.48	6.3
2	80	22.8	0.77	3.4	1.49	6.5

HIGH PANEL						
Instrument	n	Mean Conc. Value (pmol/L)	Within Run		Total	
			SD	CV(%)	SD	CV(%)
1	80	49.7	2.51	5.1	3.87	7.8
2	80	48.2	2.36	4.9	4.09	8.5

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

DILUTION LINEARITY/RECOVERY

The AxSYM Active-B12 assay is designed to have a mean recovery of $100 \pm 20\%$ when analyzing specimens diluted in AxSYM Active-B12 Low Control.

Three separate pools of high HoloTC specimens were diluted with AxSYM Active-B12 Low Control according to NCCLS document EP6-A.³⁶ In a representative study, the concentrations of HoloTC were determined using the AxSYM Active-B12 assay, and the percent recovery was calculated. The observed mean recovery was 109.3% (Observed range 101.1% - 128.0%).

ANALYTICAL SENSITIVITY

The AxSYM Active-B12 assay is designed to have a mean analytical sensitivity of ≤ 1 pmol/L.

Analytical sensitivity is defined as the concentration at two standard deviations above the AxSYM Active-B12 Standard Calibrator A (0.0 pmol/L) and represents the lowest concentration of HoloTC that can be distinguished from zero. In a representative study, the analytical sensitivity was evaluated by repeated testing ($n = 12$ runs in replicates of 10 across two reagent lots) of the AxSYM Active-B12 Standard Calibrator A. In this study, the mean analytical sensitivity was calculated as 0.08 pmol/L (observed range 0.03 to 0.14 pmol/L).

ANALYTICAL SPECIFICITY

The AxSYM Active-B12 assay was evaluated for analytical specificity in a study where cross-reactivity with B₁₂ binding proteins transcobalamin and haptocorrin was measured by the assay. 500 pmol/L transcobalamin was added to line diluent and 5000 pmol/L haptocorrin was added to Calibrator A and then assayed (n = 5 replicates). Data from this study are summarized in the following table.*

Cross-reactant	Cross-reactant Concentration (pmol/L)	% Cross-reactivity ^a
Transcobalamin	500	Not detectable ^b
Haptocorrin	5000	Not detectable ^b

$$^a \text{ \% Cross Reactivity} = \frac{\text{HoloTC (pmol/L)}}{\text{Cross-reactant added (pmol/L)}} \times 100$$

^b HoloTC results below the sensitivity of the assay.

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

CARRYOVER

In a representative study, carryover from a sample with a high HoloTC value (estimated to be 641.2 pmol/L) to an adjacent sample of AxSYM Active-B12 Standard Calibrator A (0.0 pmol/L) was determined to be below the analytical sensitivity of the assay.

INTERFERENCE

The AxSYM Active-B12 assay is designed to have a mean potential interference from bilirubin, hemoglobin, triglycerides, red blood cells, rheumatoid factor and total protein of ≤ 10% at the levels indicated in the following table* as confirmed by a study based on guidance from the Clinical and Laboratory Standards Institute document EP7-A2.³⁷

Interfering Substance	Interfering Substance Concentration
Bilirubin	0.4 mg/mL
Hemoglobin	5 mg/mL
Triglycerides	15 mg/mL
Red Blood Cells	0.4 %
Rheumatoid Factor	500 IU/mL
Total Protein	95 g/L

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

METHOD COMPARISON

A study was performed to compare the AxSYM Active-B12 assay to a commercially available radioimmunoassay for the measurement of HoloTC (Comparison Assay). The results from the Passing-Bablok³⁸ linear regression analysis are summarized in the following table:*

AxSYM Active-B12 vs. Comparison Assay

Regression Method	Specimen Type	n	Correlation Coefficient	Intercept (95% CI ^a)	Slope (95% CI ^a)
Passing-Bablok ^b	Serum	204	0.90	2.1 (-0.91 to 4.84)	1.02 (0.95 to 1.10)

Sample Range (AxSYM): 8.9 - 123.3 pmol/L.

Sample Range (Comparison Assay): 7.1 - 144.3 pmol/L.

^a Confidence Interval (CI).

^b A linear regression method with no special assumptions regarding the distribution of the samples and measurement errors.

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

BIBLIOGRAPHY

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