Abbott AXSYM SYSTEM

CE

Homocysteine

IVD REF 5F51-20

ABBL143/R7

HOMOCYSTEINE

Customer Service
United States: 1-877-4ABBOTT
International: Call your Abbott Representative

This package insert must be read carefully prior to 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.

WARNING: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Specimens from patients taking methotrexate, carbamazepine, phenytoin, nitrous oxide or 6-azauridine triacetate may have elevated levels of homocysteine due to their effect on the metabolic pathway. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this assay package insert.

Key to symbols used

REF	List Number	LOT	Lot Number
IVD	*In Vitro* Diagnostic Medical Device		Expiration Date
2°C 8°C	Store at 2-8°C	CAL A	Calibrator (A-F)
		CONTROL L	Control Low, Medium, High (L, M, H)
15°C 30°C	Store at 15-30°C		
		REAGENT PACK	Reagent Pack
	CAUTION: Consult accompanying documents	REACTION VESSELS	Reaction Vessels
		SAMPLE CUPS	Sample Cups
	Manufacturer		Consult instructions for use

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

NAME

AxSYM Homocysteine

INTENDED USE

The AxSYM Homocysteine assay is a Fluorescence Polarization Immunoassay (FPIA) for the quantitative measurement of total L-homocysteine in human serum or plasma on the AxSYM System. Homocysteine values can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

SUMMARY AND EXPLANATION OF THE TEST

Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine is exported into plasma where it circulates, mostly in its oxidized form, bound to plasma proteins as a protein-HCY mixed disulfide with albumin.[1-4] Smaller amounts of reduced homocysteine and the disulfide homocystine (HCY-SS-HCY) are present. Total homocysteine (tHCY) represents the sum of all HCY species found in serum or plasma (free plus protein bound).

Homocysteine is metabolized to either cysteine or methionine. In the vitamin B_6 dependent trans-sulphuration pathway, homocysteine is irreversibly catabolized to cysteine. A major part of homocysteine is remethylated to methionine, mainly by the folate and cobalamin-dependent enzyme methionine synthase. Homocysteine accumulates and is excreted into the blood when these reactions are impaired.[2,4]

Severely elevated concentrations of total homocysteine are found in subjects with homocystinuria, a rare genetic disorder of the enzymes involved in the metabolism of homocysteine. Patients with homocystinuria exhibit mental retardation, early arteriosclerosis and arterial and venous thromboembolism.[1,5] Other less severe genetic defects which lead to moderately elevated levels of total homocysteine are also found.[6-8]

Epidemiological studies have investigated the relationship between elevated homocysteine concentrations and cardiovascular disease (CVD). A meta-analysis of 27 of these studies, including more than 4000 patients, estimated that a 5 µmol/L increase in total homocysteine was associated with an odds ratio for coronary artery disease (CAD) of 1.6 (95% confidence interval [CI], 1.4 to 1.7) for men and 1.8 (95% CI, 1.3 to 1.9) for women; the odds ratio for cerebrovascular disease was 1.5 (95% CI, 1.3 to 1.9). The risk associated with a 5 µmol/L increase in total homocysteine was the same as that associated with 0.5 mmol/L (20 mg/dL) increase in cholesterol. Peripheral arterial disease also showed a strong association.[9]

Hyperhomocysteinemia, elevated levels of homocysteine, can be associated with an increased risk of cardiovascular disease. There have also been many published reports of prospective studies on the relationship between hyperhomocysteinemia and risk of cardiovascular disease in men and women who were initially healthy. Endpoints were based on a cardiovascular event such as acute myocardial infarction, stroke, coronary artery disease, or mortality. The results of eleven of these nested case-control studies reviewed by Cattaneo[10] were equivocal where five of the studies support the association with risk and six do not. More recently, homocysteine levels were determined in a prospective study of post-menopausal women who participated in the Women's Health Study. Specimens from 122 women, who subsequently developed cardiovascular events, were analyzed with the IMx Homocysteine assay and compared to a control group of 244 women who were matched as to age and smoking status. The women in the control group remained free of disease during a three year follow-up period. The results demonstrated that post-menopausal women who developed cardiovascular events had significantly higher baseline homocysteine levels. Those with levels in the highest quartile had a two-fold increase in risk of any cardiovascular event. Elevated baseline homocysteine levels were shown to be an independent risk factor.[11] Also, homocysteine levels were determined in 1,933 elderly men and women from the Framingham Heart Study cohort and demonstrated that elevated levels of homocysteine are independently associated with increased rates of all-cause and cardiovascular disease mortality.[12]

Patients with chronic renal disease experience an excess morbidity and mortality due to arteriosclerotic CVD. Elevated concentration of total homocysteine is a frequently observed finding in the blood of these patients. Although they may lack some of the vitamins involved in the metabolism of homocysteine, the increased levels of total homocysteine are mainly due to impaired removal of homocysteine from the blood by the kidney.[13,14]

The following drugs may elevate levels of homocysteine: methotrexate, carbamazepine, phenytoin, nitrous oxide, and 6-azauridine triacetate. The mechanism of action affects different parts of the metabolic pathway of homocysteine.[15]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The AxSYM Homocysteine assay is based on the Fluorescence Polarization Immunoassay (FPIA) technology.

Bound HCY (oxidized form) is reduced to free HCY that is enzymatically converted to S-adenosyl-L-homocysteine (SAH) as follows:[16]

Reduction: Homocystine, mixed disulfide, and protein-bound forms of HCY in the sample are reduced to form free HCY by the use of dithiothreitol (DTT).

HCY-SS-HCY (Homocystine) + R1-SS-HCY (R1 = thiol residue) + Protein-SS-HCY —DTT→ HCY

Enzymatic Conversion: Free HCY is converted to SAH by the use of SAH hydrolase and excess adenosine.

HCY + Adenosine —SAH-Hydrolase→ SAH

Under physiological conditions, SAH hydrolase converts SAH to homocysteine. Excess adenosine in the Pretreatment Solution drives the conversion of HCY to SAH by the recombinant SAH Hydrolase.

The AxSYM Homocysteine reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM Homocysteine Reagents required for one test are pipetted by the sampling probe into various wells of a Reaction Vessel (RV).
- Sample, Pretreatment Solution, Solution 4 (Line Diluent), and SAH Hydrolase are pipetted into one well of the RV to make up the predilution mixture.

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 predilution mixture, Antibody, and Solution 4 (Line Diluent) are transferred to the cuvette of the RV.
- Tracer, Solution 4, and a second aliquot of the predilution mixture are transferred to the cuvette.
- SAH and labeled Fluorescein Tracer compete for the sites on the monoclonal antibody molecule.
- The intensity of polarized fluorescent light is measured by the FPIA optical assembly.

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

REAGENTS

REAGENT PACK, 100 TESTS

AxSYM Homocysteine Reagent Pack, 100 Tests (5F51-20)*

- 1 Bottle (15.0 mL) S-adenosyl-L-cysteine Fluorescein Tracer in phosphate buffer with protein (bovine) stabilizer. Preservative: sodium azide.
- 1 Bottle (12.4 mL) S-adenosyl-L-homocysteine hydrolase (recombinant) in phosphate buffer with protein (bovine) stabilizer. Preservatives: sodium azide and antimicrobial agents.
- 1 Bottle (15.6 mL) Anti-S-adenosyl-L-homocysteine (mouse monoclonal) in phosphate buffer with protein (porcine) stabilizer. Preservative: sodium azide.
- 1 Bottle (15.3 mL) Pretreatment Solution containing dithiothreitol (DTT) and adenosine in citric acid.

  NOTE: This FPIA assay utilizes a reagent pack with 4 bottles.

* 5F51-99 includes an AxSYM Homocysteine Reagent Pack (100 tests) and Reaction Vessels (100 each). 5F51-20 includes these items for international shipment.

CALIBRATORS

Homocysteine Calibrators (9F84-01)

1 Bottle (3.5 mL) Homocysteine Calibrator A contains phosphate buffer. 5 Bottles (2.5 mL each) of Homocysteine Calibrators B-F contain gravimetrically prepared S-adenosyl-L-homocysteine in phosphate buffer at the following concentrations:

Bottle	Homocysteine Concentration (µmol/L)
CAL A	0.0
CAL B	2.5
CAL C	5.0
CAL D	10.0
CAL E	20.0
CAL F	50.0

Preservative: sodium azide.

The Homocysteine Calibrators are manufactured gravimetrically with S-adenosyl-L-homocysteine (SAH) in phosphate buffer and tested for acceptance using internal reference standards.

CONTROLS

Homocysteine Controls (9F84-10)

3 Bottles (2.5 mL each) of Homocysteine Controls contain L-homocystine in processed human serum (nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti HCV or HCV RNA) and phosphate buffer to yield the following concentration ranges:

Bottle	Homocysteine Concentration (µmol/L)	Range (µmol/L)
CONTROL L	7.0	5.25 - 8.75
CONTROL M	12.5	10.00 - 15.00
CONTROL H	25.0	20.00 - 30.00

Preservative: sodium azide.

The AxSYM Homocysteine default result unit is µmol/L. The alternate result unit (µg/mL) may be selected for reporting results (Assay Parameter 45). The conversion factor used by the AxSYM System is 0.1352.

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 4 (Line Diluent) (8A46)

SOLUTION 4 LINE DILUENT 1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M Phosphate buffer. Preservatives: sodium azide and antimicrobial agent.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

SAFETY PRECAUTIONS

CAUTION: This product contains human sourced infectious and/or potentially infectious components. Refer to the REAGENTS section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.

- Some components of this product contain sodium azide. For a specific listing, refer to the REAGENTS section of this package insert. The Antibody, Fluorescein Tracer, Calibrators and Controls are 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.

HANDLING PRECAUTIONS

- Do not use the Reagent Pack beyond the expiration date or a maximum of 336 cumulative hours on-board the AxSYM System.
- Do not mix reagents from different Reagent Packs.
- The Fluorescein Tracer, Reagent Bottle 1, is sensitive to light and is packaged in a black bottle to protect the solution.

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 Homocysteine Reagent Pack and Homocysteine Calibrators and Controls must be stored at 2-8°C. They may be used immediately after removing them from the refrigerator. Calibrators and controls should be returned to 2-8°C storage immediately after use. Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM Homocysteine Reagent Pack may be on-board the AxSYM System for a maximum of 336 cumulative hours; for example, 42 eight hour shifts. After 336 hours, the reagent pack must be discarded. Refer to the AxSYM System Operations Manual, Sections 2, 5, and Appendix C, for further information on tracking on-board time.

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

INSTRUMENT PROCEDURE

Assay File Installation

The AxSYM Homocysteine Assay File must be installed on the AxSYM System from the software disk, No. 7G53-01 or higher, prior to performing AxSYM Homocysteine assays. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

NOTE: AxSYM Homocysteine must only be run with AxSYM System(s) software version 3.60 or higher.

AxSYM Homocysteine Assay Parameters

The default values for the assay parameters used for the AxSYM Homocysteine 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 Parameters screen. Press PRINT to print the assay parameters.

	Assay Parameters
1	Long Assay Name (English): Homocysteine
6	Abbrev Assay Name (English): tHCY
11	Assay Number: 345
12	Assay Version: *
13	Calibration Version: *
14	Assay File Revision: *
15	Assay Enabled > ON
17	Assay Type: FPIA
18	Standard Cal Reps > 2
21	CAL A Concentration: 0.00
22	CAL B Concentration: 2.50
23	CAL C Concentration: 5.00
24	CAL D Concentration: 10.00
25	CAL E Concentration: 20.00
26	CAL F Concentration: 50.00
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > µmol/L
46	Selected Results Decimal Places > 2
62	Blank I-Max background intensity: *
63	Min Tracer-Min net intensity: *
75	Low Extreme Value > 0.80
76	High Extreme Value > 500.00
91	Low Range Undiluted: 0.00
92	High Range Undiluted: 50.00
96	Low Range Dil1 : 40.00
97	High Range Dil1 : 500.00

NOTE: Parameter 45 can be edited to the alternate result unit, µg/mL.

It is recommended that the General Configuration Parameter, Release Mode, be set to the "Manual" or "Hold" release mode to ensure that all flagged results are reviewed prior to reporting assay results. Refer to the AxSYM System Operations Manual, Section 2, for a detailed description of Instrument Procedures. If the General Configuration Parameter, Release Mode, is configured to the "Automatic" release mode, ensure that all flagged results are reviewed prior to reporting assay results.

Refer to the AxSYM System Operations Manual for a detailed description of Instrument Procedures. For details on Automatic Sample Retest Configuration, refer to the AxSYM System Operations Manual, Section 2.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Serum (including serum collected in serum separator tubes) and plasma (collected in tripotassium EDTA or lithium heparin) may be used in the AxSYM Homocysteine assay. Other anticoagulants have not been validated with the AxSYM Homocysteine assay.

To minimize increases in Homocysteine concentration from synthesis by red blood cells, process specimens as follows:

- Place all specimens (serum and plasma) on ice after collection and prior to processing. Serum may clot more slowly and the volume of serum may be reduced.[15]
- All specimens may be kept on ice for up to 6 hours prior to separation by centrifugation.[15]
- Separate red blood cells from serum or plasma by centrifugation and transfer to an AxSYM Sample Cup or other clean container.

NOTE: Specimens not placed on ice immediately may exhibit a 10-20% increase in concentration.[17]

- If the assay will be performed within 2 weeks after collection, the sample should be stored at 2-8°C. If testing will be delayed more than 2 weeks, the specimen should be stored frozen at -20°C or colder. Samples have been shown to be stable at -20°C for 8 months.
- The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify the correct sample type(s) is (are) used in the AxSYM Homocysteine assay.
- To minimize the effects of evaporation, all samples (patient specimens, controls, and calibrators) 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 more detailed discussion of on-board sample storage constraints.
- Inspect all samples for bubbles. Remove bubbles prior to analysis.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter.
- Mix specimens **thoroughly** after thawing by LOW speed vortexing or by gently inverting to ensure consistency in the results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity should be centrifuged before testing.
- 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. Prior to shipment, specimens must be removed from the clot or red blood cells. Specimens may be shipped at 2-8°C.

SAMPLE VOLUME

The sample volume required to perform a single AxSYM Homocysteine test on the AxSYM System varies depending on the type of sample container used. For sample cups, a ROUTINE test requires 150 µL and a STAT test requires 108 µL. For every additional AxSYM Homocysteine test performed (ROUTINE or STAT) from the same sample container, an additional 58 µL of sample is required.

The sample cup minimum volumes for both STAT and ROUTINE tests are calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is (are) ordered and printed on the Orderlist Report. When using Host Order Query, the Order screen information and the Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query option.

If the assay is configured for auto retest, the additional sample volume needed for the retest will not be displayed on the order screen at the time the test(s) is(are) ordered. Therefore, the total sample volume should include an additional 58 µL of sample. Refer to the AxSYM System Operations Manual, Section 2, for details on Automatic Sample Retest Configuration.

Refer to the AxSYM System Operations Manual, Section 5, for sample volume requirements in primary or aliquot tubes and calibrator/control requirements for multiple reagent lots.

AxSYM HOMOCYSTEINE PROCEDURE

Materials Provided

- 5F51-99 (USA) or 5F51-20 (International) — AxSYM Homocysteine Reagent Kit containing: AxSYM Homocysteine **REAGENT PACK** 100 **REACTION VESSELS**

Materials Required but not Provided

- AxSYM System
- 9F84-10 — Homocysteine Controls
- 9F84-01 — Homocysteine Calibrators
- 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 Order screen

CAUTION:

- Homocysteine Calibrators and Controls should be mixed by gentle inversion 3 to 5 times prior to use.
- When manually dispensing sample into sample cups, verify that dispensing equipment does not introduce cross contamination and 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 contain detailed steps for performing assay calibration and sample testing procedures.

Prior to ordering tests, confirm that the System inventory of Reaction Vessels (RVs), bulk solutions, and waste levels are acceptable.

The Orderlist Report contains sample placement information and sample cup volume 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 Reaction Vessels (RVs).
- An "Error Code 5066 Matrix Cell not detected, trap door, processing center" may be displayed when the instrument homes the motors. If performing only FPIA (and/or REA) assays, select **OK** to proceed with testing.
- 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. Tests in process will be terminated and must be repeated.
- When testing is completed, it is recommended that samples and the AxSYM Homocysteine Reagent Pack are removed from the Sampling Center to maximize the on-board reagent pack use. Store at 2-8°C.

SPECIMEN DILUTION PROCEDURES

Patient specimens with homocysteine values exceeding 50 µmol/L (HIGH RANGE, Assay Parameter 92) are flagged with the code "> 50.00." To quantitate the concentration of these specimens, perform either the Automated Dilution or Manual Dilution Protocol.

The Blank I-max background intensity, assay parameter 62, is set at 45,000.00. Specimens with a background intensity greater than 45,000.00 will be followed by Exception: 1065. The sample may be repeated by performing the Manual Dilution Procedure.

Automated Dilution Protocol

The Automated Dilution Protocol is provided to assist in quantitating test results greater than 50 µmol/L and up to 500 µmol/L. The AxSYM System performs a 1:10 dilution of the specimen using one RV. The AxSYM System automatically calculates the concentration of the diluted specimen and reports the result.

If the assay is configured for auto dilution, an additional 58 µL of sample volume needed for the dilution should be included in the sample container when ordering tests.

Refer to the AxSYM System Operations Manual, Section 5, for additional information on ordering specimen dilutions.

Manual Dilution Protocol

Patient specimens with homocysteine concentrations reported as greater than 50 µmol/L may be diluted using a suggested manual dilution of 1:10. To make this dilution, add 15 µL of the patient sample to 135 µL of Calibrator A. The dilution should be performed so that the diluted sample result reads greater than 2.5 µmol/L. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

$$\text{Final Specimen Concentration} = \text{Printed Concentration} \times \text{Manual Dilution Factor}$$

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of Dilution Reagent)}}{\text{Volume of Specimen}}$$

QUALITY CONTROL PROCEDURES

CALIBRATION

The AxSYM Homocysteine assay must be calibrated using a Standard Calibration (6-point) procedure.

Standard Calibration

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

Once the AxSYM Homocysteine 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.

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.

Operator Verification

An acceptable AxSYM Homocysteine calibration should meet the following criteria:

a) Polarization Error (PERR) -2.25 to +2.25 for all calibrators.
b) Root Mean Squared Error (RMSE) less than or equal to 1.75.
c) All controls are within the acceptable ranges.

NOTE: PERRs and RMSEs are to be used as guidelines only. If controls are within specified ranges, the calibration curve is acceptable.

QUALITY CONTROL

The recommended control requirement for an AxSYM Homocysteine assay is a single sample of all Homocysteine control levels tested once every 24 hours each day of use. Controls may be placed in any position in the Sample Carousel.

Ensure that assay control values are within the concentration ranges specified in this package insert. Refer to the **REAGENTS**, CONTROLS section of this package insert for Homocysteine control ranges.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When a homocysteine 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. Assay 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. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

Refer to the AxSYM System Operations Manual, Section 2, for further information on this parameter.

RESULTS

CALCULATION

The AxSYM Homocysteine assay utilizes a Four-Parameter Logistic Curve fit method (4PLC Y-weighted) to generate a standard calibration curve.

ALTERNATE RESULT UNIT

The default result unit for AxSYM Homocysteine is µmol/L. When selecting the alternate result unit, µg/mL, the conversion factor used by the AxSYM System is 0.1352.

Concentration in µg/mL = Concentration in µmol/L x 0.1352

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

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibody (HAMA). HAMA present in serum or plasma specimens, may interfere in immunoassays which utilize mouse monoclonal antibodies.[18,19] These specimens should not be assayed with the AxSYM Homocysteine assay.
- S-adenosyl-methionine is an antidepressant whose molecular form is similar to S-adenosyl-homocysteine. Therapeutic levels of this drug may interfere with the AxSYM Homocysteine assay.
- The following drugs may elevate levels of homocysteine: methotrexate, carbamazepine, phenytoin, nitrous oxide, and 6-azauridine triacetate. The mechanism of action affects different parts of the metabolic pathway of homocysteine.[15]
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert for additional information.

EXPECTED VALUES

Human plasma specimens from 340 apparently healthy individuals were evaluated using AxSYM Homocysteine assay. Exclusion criteria for donors were: cancer treatment, premature (younger than 45 years old) onset of heart disease (excluding hypertension) and treatment with S-adenosyl-methionine, methotrexate, carbamazepine, phenytoin, nitrous oxide, or 6-azauridine triacetate. The range of expected values is defined by the central 95% of the observations. The distribution was represented as follows:

AxSYM Homocysteine Expected Values

				Percentile	
Sex	n	Median (µmol/L)	Range (µmol/L)	2.5% (µmol/L)	97.5% (µmol/L)
Male	170	8.80	5.90 - 16.00	6.26	15.01
Female	170	6.91	3.36 - 20.44	4.60	12.44
Overall	340	8.01	3.36 - 20.44	4.72	14.05

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

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

Precision was determined as described in the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-T2.[20] Three processed human serum and buffer based panels (1, 2, and 3) were assayed in replicates of two, at two separate times per day, for 20 days, on two instruments, using two lots of reagents and a single standard calibration per instrument. Data from this study are summarized below.

PANEL 1

			Mean	Within Run		Total	
Lot	Instrument	n	µmol/L	SD	%CV	SD	%CV
1	1	80	7.99	0.358	4.5	0.368	4.6
	2	80	7.29	0.174	2.4	0.318	4.4
2	1	80	7.84	0.231	2.9	0.240	3.1
	2	80	7.62	0.192	2.5	0.389	5.1

PANEL 2

			Mean	Within Run		Total	
Lot	Instrument	n	µmol/L	SD	%CV	SD	%CV
1	1	80	13.71	0.354	2.6	0.426	3.1
	2	80	13.14	0.232	1.8	0.360	2.7
2	1	80	13.37	0.312	2.3	0.346	2.6
	2	80	13.91	0.257	1.8	0.504	3.6

PANEL 3

			Mean	Within Run		Total	
Lot	Instrument	n	µmol/L	SD	%CV	SD	%CV
1	1	80	27.43	0.494	1.8	0.561	2.0
	2	80	26.67	0.445	1.7	0.744	2.8
2	1	80	26.60	0.567	2.1	0.589	2.2
	2	80	28.17	0.397	1.4	0.724	2.6

AUTOMATED DILUTION

The Automated Dilution Protocol (1:10) was compared to a manual 1:10 protocol using 8 human specimens with homocysteine levels that were greater than Calibrator F (50 µmol/L). The manual dilution was performed with Calibrator A. One replicate each of the autodiluted and manually diluted sample were assayed on one AxSYM instrument.

Specimen	Automated Dilution (µmol/L)	Manual Dilution (µmol/L)	%Recovery*
1	63.69	65.90	96.6
2	52.91	51.60	102.5
3	96.24	91.90	104.7
4	54.93	54.10	101.5
5	70.73	70.00	101.0
6	94.86	92.50	102.6
7	73.23	70.00	104.6
8	63.61	60.60	105.0

Average %Recovery: 102.3

$$* \% \text{ Recovery} = \frac{\text{Automated Dilution (µmol/L)}}{\text{Manual Dilution (µmol/L)}} \times 100$$

DILUTION LINEARITY

Dilution linearity was evaluated by serial dilution of human specimens of known homocysteine concentrations. All specimens were diluted with Calibrator A.

Specimen	Dilution Factor	Expected (µmol/L)	Mean Observed (µmol/L)	%Recovery*
A	1:2	46.96	46.96	–
	1:4	23.48	23.67	100.8
	1:8	11.74	11.63	99.1
	1:16	5.87	5.44	92.7
	1:32	2.94	2.54	86.4
B	Undiluted	49.45	49.45	–
	1:2	24.73	25.07	101.4
	1:4	12.36	12.63	102.2
	1:8	6.18	6.25	101.1
	1:16	3.09	2.95	95.5
C	1:2	35.42	35.42	–
	1:4	17.71	17.95	101.4
	1:8	8.86	8.87	100.1
	1:16	4.43	4.03	91.0
D	Undiluted	35.27	35.27	–
	1:2	17.64	17.07	96.8
	1:4	8.82	8.62	97.7
	1:8	4.41	4.33	98.2

$$* \% \text{ Recovery} = \frac{\text{Mean Observed (µmol/L)}}{\text{Expected (µmol/L)}} \times 100$$

SENSITIVITY

The sensitivity of the AxSYM Homocysteine assay was calculated to be ≤ 0.8 µmol/L by testing Calibrator A (n = 24 runs; in replicates of 10). Sensitivity is defined as the concentration at two standard deviations from the mean of the AxSYM Homocysteine Calibrator A and represents the lowest measurable concentration of Homocysteine that can be distinguished from zero.

SPECIFICITY

Cross-reactivity was tested for compounds whose chemical structure or concurrent usage may potentially interfere with the AxSYM Homocysteine assay. Solutions of the compounds were added into plasma to give the concentrations indicated in the table below. These concentrations are more than twenty-fold the physiological concentrations. Results are summarized as follows:

Compound	Concentration (mM)	%Cross-Reactivity
S-Adenosyl-L-Methionine	0.5	1.28
L-Cysteine	100.0	0.03
L-Cystathionine	0.5	0.08
Adenosine	5.0	1.15
Glutathione	100.0	0.01
DL-Homocysteine Thiolactone	0.25	-0.19

INTERFERENCE

Potential interference from bilirubin, triglycerides, total protein, hemoglobin, and red blood cells was evaluated in the AxSYM Homocysteine assay. The AxSYM Homocysteine assay demonstrated < 10% interference in the presence of each of the following potentially interfering substances:

Interfering Substance	Concentration
Bilirubin	20.3 mg/dL
Triglycerides	6439 mg/dL
Total Protein	11.0 g/dL
Hemoglobin	1000 mg/dL
Red Blood Cells	5.0%

ACCURACY BY CORRELATION

The AxSYM Homocysteine assay was compared to the IMx Homocysteine assay. The results of the linear regression analyses follow.

Abbott AxSYM Homocysteine vs. IMx Homocysteine

Regression Analysis	n	Y-Intercept	Slope	Correlation Coefficient
Least Squares	300	-0.56	1.04	0.985
Passing-Bablok*	300	-0.80	1.08	0.985

* A linear regression method with no special assumptions regarding distribution of specimens and measurement errors.[21]

Specimen values ranged from 6.43 to 48.70 µmol/L with the AxSYM Homocysteine assay and 6.83 to 48.08 µmol/L with the IMx Homocysteine assay.

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ABBOTT
Max-Planck-Ring 2
65205 Wiesbaden
Germany
+49-6122-580

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ABBOTT
Diagnostics Division

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