Abbott AXSYM SYSTEM

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Myoglobin

REF 7K48

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B7K480

Read Highlighted Changes
Revised August, 2010

MYOGLOBIN

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

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	STANDARD CAL A	Standard Calibrator (A-F)
IVD	*In Vitro* Diagnostic Medical Device	CONTROL L	Control Low, Medium, High (L, M, H)
LOT	Lot Number	REAGENT PACK	Reagent Pack
	Expiration Date	REACTION VESSELS	Reaction Vessels
2°C 8°C	Store at 2-8°C	SAMPLE CUPS	Sample Cups
15°C 30°C	Store at 15-30°C		Consult instructions for use
	Caution	CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
	Manufacturer		

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

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WARNING: Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the AxSYM Myoglobin assay. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this assay package insert.

NAME

AxSYM Myoglobin

INTENDED USE

AxSYM Myoglobin is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of myoglobin in human serum or plasma on the AxSYM System.

SUMMARY AND EXPLANATION OF THE TEST

Myoglobin is a tightly folded, globular heme-protein located in the cytoplasm of both skeletal and cardiac muscle cells. Its function is to store and supply oxygen to muscle cells. The molecular weight of myoglobin is approximately 17,800 daltons.[1,2] The relatively low molecular weight and the location of storage accounts for the rapid release from damaged muscle cells and earlier rises in concentration measured above baseline in blood as compared to other cardiac markers.[1,3,4]

In acute ischemic heart disease, such as Acute Myocardial Infarction (AMI), a temporal pattern of increased release of myoglobin into the blood stream is observed. The serum or plasma myoglobin level will start to show an increase between 2-4 hours after an AMI has occurred, peaking at approximately 8-10 hours, and returning to baseline after 24 hours. Measurement of myoglobin between 2-12 hours after an AMI can be a good adjunct to electrocardiology in improving the efficiency of early diagnosis of AMI.[1,5,6] Monitoring the myoglobin levels can also help in evaluating the success of thrombolytic therapy.[6,7]

Since myoglobin is present in both cardiac and skeletal muscle, any damage to either of these muscle types results in its release into the blood stream. Serum levels of myoglobin have been shown to elevate under the following conditions: skeletal muscle damage, skeletal muscle or neuromuscular disorders, cardiac bypass surgery, renal failure, strenuous exercise, etc.[2,5,8] Therefore, the utilization of an increase in serum myoglobin has to be used in conjunction with other aspects of the patient assessment in order to aid in the diagnosis of an AMI. Myoglobin may also rise moderately above the reference range in chronic ischemic heart disease (i.e. unstable angina).[2] For diagnostic purposes, the AxSYM Myoglobin assay results should be used in conjunction with other data; e.g., other clinical testing, ECG, symptoms, clinical observations, etc.[8]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The AxSYM Myoglobin assay is based on Microparticle Enzyme Immunoassay (MEIA) technology. The AxSYM Myoglobin Reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM Myoglobin Reagents required for one test are pipetted by the Sampling Probe into various wells of a Reaction Vessel (RV).
- Sample and anti-Myoglobin coated Microparticles are combined in a well of the RV. The myoglobin in the sample binds to the antibody coated on the microparticles, forming an antigen-antibody complex.
- The anti-Myoglobin: Alkaline Phosphatase Conjugate is pipetted into a second 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 sample/microparticle mixture, containing the antigen-antibody complex bound to the Microparticles, is transferred to the Matrix Cell. The antigen-antibody-microparticle complex remains on the surface of the Matrix Cell while unbound material is removed by a Matrix Cell Wash.
- The Conjugate is then dispensed onto the Matrix Cell and allowed to bind to the myoglobin of the antigen-antibody complex, forming an antibody-antigen-antibody complex.
- The Matrix Cell is washed to remove unbound materials.
- The substrate, 4-Methylumbelliferyl Phosphate, is added to the Matrix Cell and the fluorescent product formed is measured by the MEIA optical assembly. The rate of formation of the fluorescent product is directly related to the amount of myoglobin in the sample.

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

REAGENTS

REAGENT PACK, 100 TESTS

AxSYM Myoglobin Reagent Pack (7K48-20)*

- 1 Bottle (14.6 mL) Anti-Myoglobin (mouse, monoclonal) coated Microparticles in TRIS buffer. Preservative: Antimicrobial Agent. (Reagent Bottle 3)
- 1 Bottle (13.4 mL) Anti-Myoglobin (caprine): Alkaline Phosphatase Conjugate in TRIS buffer with protein (piscine, bovine) stabilizers. Preservative: Sodium Azide. (Reagent Bottle 2)

* 7K48-20 includes an AxSYM Myoglobin Reagent Pack (100 tests), Reaction Vessels (100 each), and Matrix Cells (100 each).

CALIBRATORS

AxSYM Myoglobin Standard Calibrators (7K48-01)

6 Bottles (2 mL each) of AxSYM Myoglobin Standard Calibrators.

Calibrator A is TRIS buffer with protein (bovine) stabilizers. Calibrators B through F contain myoglobin (human) in TRIS buffer with protein (bovine) stabilizers to yield the following concentrations:

Bottle	Myoglobin Concentration* (ng/mL, µg/L)
STANDARD CAL A	0
STANDARD CAL B	25
STANDARD CAL C	100
STANDARD CAL D	300
STANDARD CAL E	500
STANDARD CAL F	1000

Preservative: Sodium Azide.

* AxSYM Myoglobin Standard Calibrators are manufactured gravimetrically using highly purified human cardiac myoglobin. They are tested by rate-match to internal reference calibrator standards prepared from highly purified human cardiac myoglobin. The myoglobin concentration of these internal reference calibrator standards is traceable to material with a protein concentration determined using a reference protein standard.

CONTROLS

AxSYM Myoglobin Controls (7K48-10)

3 Bottles (8 mL each) of AxSYM Myoglobin Controls contain myoglobin (human) in TRIS buffer with protein (bovine) stabilizers to yield the following concentrations:

Bottle	Myoglobin Concentration (ng/mL, µg/L)	Range (ng/mL, µg/L)
CONTROL L	50	33.6 - 66.5
CONTROL M	150	100.6 - 199.4
CONTROL H	400	268.4 - 531.6

Preservative: Sodium Azide.

The AxSYM Myoglobin default result unit is ng/mL. An alternate result unit (µg/L) may be selected for reporting results (Assay Parameter 45). The conversion factor used by the AxSYM System is 1.0.

The calibrators and controls are matched to an Abbott internal reference standard. This internal reference standard is manufactured by dilution using a human cardiac myoglobin stock solution containing human cardiac myoglobin (purity by electrophoresis ≥ 99%) at each concentration level.

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) (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

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

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

SAFETY PRECAUTIONS

- **CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, 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, 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.[9] Biosafety Level 2[10] or other appropriate biosafety practices[11,12] should be used for materials that contain or are suspected of containing infectious agents.
- The Calibrators B-F contain human myoglobin, donor units which have been tested and found to be nonreactive for Hepatitis B, Hepatitis C, HIV-1 and HIV-2.
- The Controls contain human myoglobin, donor units which have been tested and found to be nonreactive for Hepatitis B, Hepatitis C, HIV-1 and HIV-2.
- This product contains sodium azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

HANDLING PRECAUTIONS

- **AxSYM Myoglobin Reagents are susceptible to bubbles/foaming and require inspection and removal of bubbles before loading. Refer to the AxSYM System Operations Manual, Section 9.**
- **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.**
- Do not use 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.

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 Myoglobin Reagent Pack and the AxSYM Myoglobin Calibrators and Controls must be stored at 2-8°C (do not freeze). The AxSYM Myoglobin Reagent Pack may be used immediately after removing it from the refrigerator.

Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM Myoglobin 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. Refer to the AxSYM System Operations Manual, Sections 2, 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 removing it from the refrigerator. MUP 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 Myoglobin Assay File must be installed on the AxSYM System from one of the following assay disks, prior to performing AxSYM Myoglobin assays:

- 3D52-02
- 7G53-01 or higher

Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM Myoglobin Assay Parameters

The default values for the assay parameters used for the AxSYM Myoglobin 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): Myoglobin
6	Abbrev Assay Name (English): Myoglobin
11	Assay Number: 350
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: 0.0
22	Cal B Concentration: 25.0
23	Cal C Concentration: 100.0
24	Cal D Concentration: 300.0
25	Cal E Concentration: 500.0
26	Cal F Concentration: 1000.0
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > ng/mL
46	Selected Result Decimal Places > 1
92	High Range Undiluted: 1000.0
96	Low Range Dil1: 700.0
97	High Range Dil1: 10,000.0

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

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

- Human serum (including serum collected in serum separator tubes [SST]) or plasma (collected in lithium heparin, sodium heparin, and tripotassium EDTA tubes) may be used in the AxSYM Myoglobin assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes. Linear regression analysis comparing 54 plasma and SST specimens to serum specimens, demonstrated a correlation coefficient of 0.984 or greater and slope difference of 5% or less. These specimens exhibited an average percent difference of up to 14.1%. Therefore, **serial sampling for the same patient should be done with the same collection tube type.**
- 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 Myoglobin assay.
- **Ensure that complete clot formation has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.**
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter.
- Patient specimens with a cloudy or turbid appearance (possibly due to a high lipid concentration) must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer on the surface when withdrawing the specimen.
- Multiple freeze/thaw cycles should be avoided. Specimens must be mixed **thoroughly** after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.
- If testing will be delayed more than 8 hours, serum or plasma should be separated from the clot or red blood cells. Specimens may be stored for up to 1 week at 2-8°C or stored frozen (-10°C or colder) prior to being tested. Patient specimens must be mixed and centrifuged after any freeze/thaw cycle or to remove red blood cells or other particulate matter. Specimens stored frozen at -10°C or colder for 30 days did not show performance differences.
- All samples (patient specimens, controls, and calibrators) should be tested within 4 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.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of specimens and infectious substances. Specimens must be shipped frozen on dry ice or at 2-8°C with cold packs. Prior to freezing, serum or plasma specimens must be removed from the clot or red blood cells.

SAMPLE VOLUME

The sample volume required to perform a single undiluted myoglobin test on the AxSYM System varies depending on the type of sample container used. For sample cups, ROUTINE tests require 150 µL and STAT tests require 105 µL. For every additional AxSYM Myoglobin test performed (ROUTINE or STAT) from the same sample container, an additional 55 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 Orderlist screen at the time the test(s) is (are) ordered and printed on the Orderlist Report. When using Host Order Query, the Orderlist 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 Orderlist screen at the time the test(s) is (are) ordered. Therefore, the total sample volume should include an additional 55 µL of sample. Refer to the AxSYM System Operations Manual, Section 2, for details on Automatic Sample Retest Configuration.

If the assay is configured for auto dilution, an additional 55 µL of sample volume needed for the dilution should be included in the sample container when ordering tests.

To obtain the recommended volume requirements for the AxSYM Myoglobin Calibrators and Controls, hold the bottles **vertically** and dispense 4 drops of each calibrator or 4 drops of each control into each respective sample cup.

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 MYOGLOBIN PROCEDURE

Materials Provided

- 7K48-20 AxSYM Myoglobin Reagent Kit, containing:
  AxSYM Myoglobin **REAGENT PACK**
  100 **REACTION VESSELS**
  100 **MATRIX CELLS**

Materials Required but not Provided

- AxSYM System
- 7K48-10 AxSYM Myoglobin Controls
- 7K48-01 AxSYM Myoglobin Standard Calibrators
- 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:

- 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 Matrix Cells, 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).
- 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 Myoglobin 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 a myoglobin assay value exceeding 1000.0 ng/mL (HIGH RANGE, assay parameter 92) are flagged with the code "> 1000.0 ng/mL". To quantitate the concentration of these specimens, perform the Automated Dilution Protocol.

Automated Dilution Protocol

The Automated Dilution Protocol is provided to assist in quantitating test results greater than 1000.0 ng/mL and up to 10,000.0 ng/mL. The AxSYM System performs a 1:10 dilution of the unknown specimen using one Reaction Vessel. 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 55 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

An additional manual dilution of 1:10 is suggested for patient specimens with myoglobin concentrations greater than 10,000.0 ng/mL. For example, add 30 µL of the patient specimen to 270 µL of AxSYM Solution 4 (Line Diluent). This dilution should be performed so that the diluted test results read greater than the level of the Calibrator B (25 ng/mL). The concentration reported by the AxSYM System must be multiplied by the manual dilution factor (e.g., 10) 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 Myoglobin Assay must be calibrated using a Standard Calibration (6-point) procedure.

Standard Calibration

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

Once the AxSYM Myoglobin 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 recommended control requirement for an AxSYM Myoglobin assay is a single sample of all myoglobin control levels tested once every 24 hours each day of use. 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.

To achieve maximum on-board reagent stability, more frequent use of controls may be required to monitor reagent performance within the same lot.

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

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When a Myoglobin 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.

Fluorescence Background Acceptance Criteria

Quality Control with regard to the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64, Max Intercept-Max MUP intercept, each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the result is invalid. The test request will be moved to the Exceptions List where it will appear with the message "1064 Invalid test result, intercept too high" and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10, when this error message is obtained.

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

RESULTS

CALCULATION

AxSYM Myoglobin utilizes a Four-Parameter Logistic Curve fit method (4PLC, Y-weighted) to generate a Standard Calibration curve. Refer to the AxSYM System Operations Manual, Appendix F, for further information.

ALTERNATE RESULT UNIT

The default result unit for AxSYM Myoglobin is ng/mL. When selecting the alternate result unit, µg/L, the conversion factor used by the AxSYM System is 1.0. The conversion formula to change to the alternate result unit is:

$$\text{ng/mL} \times 1.0 = \text{µg/L}$$

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.

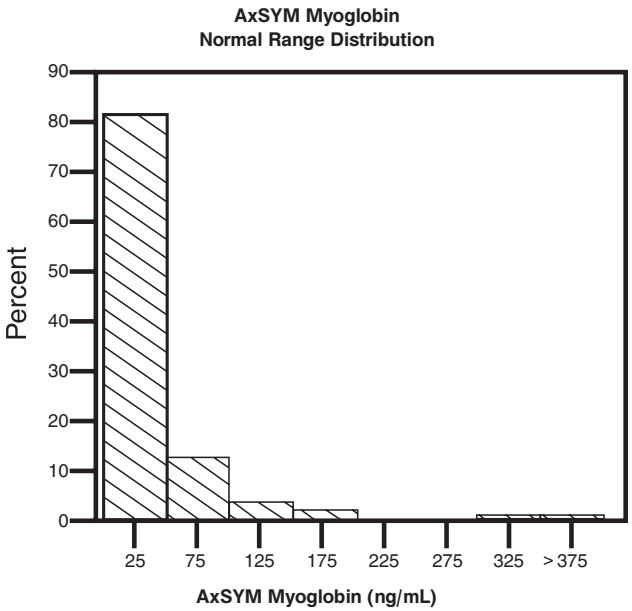
LIMITATIONS OF THE PROCEDURE

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[13] Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[13,14] These specimens should not be assayed with the AxSYM Myoglobin assay.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert for additional information.

For diagnostic purposes, the AxSYM Myoglobin results should be used in conjunction with other data; e.g., other clinical testing, ECG, symptoms, clinical observations, etc.

EXPECTED VALUES

Human serum specimens from 203 apparently healthy, prescreened blood donors were evaluated using the AxSYM Myoglobin assay. The normal range obtained by testing these specimens is ≤ 116.3 ng/mL (97.5 percentile). The distribution is represented in the histogram below:



It is recommended that each laboratory establish its own expected values, which may be unique to the population it serves depending on geographical, patient, dietary, or environmental factors, and to reflect current practice and criteria. Serial sampling may be required to detect elevated levels. Any condition resulting in skeletal or cardiac muscle damage can potentially increase myoglobin levels above the expected value.

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

The AxSYM Myoglobin assay is designed to have a precision of ≤ 10% (total CV). Precision was determined as described in the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-T2.[15] Three buffer based panels (1, 2, and 3) and three processed human serum based panels (4, 5, and 6*) were assayed in replicates of two, at two separate times per day, for 20 days, on 4 instruments, using a single lot of reagents and a single standard calibration per instrument. Data from this study are summarized below.**

* Panel 6 is a [1:10] dilution of panel 5 using the automated dilution protocol.

PANEL 1

Instrument	n	Mean Conc. Value (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
1	80	49.3	1.54	3.1	2.04	4.1
2	80	52.6	2.12	4.0	2.16	4.1
3	80	47.4	3.11	6.6	3.23	6.8
4	80	47.9	2.31	4.8	2.46	5.1

PANEL 2

Instrument	n	Mean Conc. Value (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
1	80	143.8	4.67	3.2	5.57	3.9
2	80	147.5	5.92	4.0	6.83	4.6
3	80	143.4	4.34	3.0	6.05	4.2
4	80	149.5	4.92	3.3	6.53	4.4

PANEL 3

Instrument	n	Mean Conc. Value (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
1	80	363.1	13.77	3.8	16.53	4.6
2	80	360.6	20.48	5.7	23.65	6.6
3	80	375.8	17.92	4.8	18.70	5.0
4	80	390.0	18.79	4.8	21.12	5.4

PANEL 4

Instrument	n	Mean Conc. Value (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
1	80	94.2	2.63	2.8	3.85	4.1
2	80	96.2	3.29	3.4	4.30	4.5
3	80	90.8	3.20	3.5	4.42	4.9
4	80	94.8	3.82	4.0	4.53	4.8

PANEL 5

Instrument	n	Mean Conc. Value (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
1	80	704.9	50.53	7.2	55.11	7.8
2	80	713.4	55.32	7.8	70.28	9.9
3	80	710.5	41.22	5.8	49.94	7.0
4	80	720.4	42.46	5.9	58.87	8.2

PANEL 6

Instrument	n	Mean Conc. Value (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
1	80	643.3	22.29	3.5	24.97	3.9
2	80	666.7	38.22	5.7	39.94	6.0
3	80	597.7	25.76	4.3	31.23	5.2
4	80	628.6	30.23	4.8	31.35	5.0

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

RECOVERY

Myoglobin was used to supplement three human serum specimens at three concentration levels. The concentration of myoglobin was determined using the AxSYM Myoglobin assay and the resulting percent recovery was calculated.

Specimen	Endogenous Myoglobin (ng/mL)	Myoglobin Added (ng/mL)	Myoglobin Observed (ng/mL)	% Recovery[a]
1	38.4	38.0	67.1	87.8
	38.4	67.0	98.1	93.1
	38.4	221.0	265.8	102.5
2	22.3	38.0	51.0	84.6
	22.3	67.0	82.5	92.4
	22.3	221.0	293.9	120.8
3	43.4	38.0	72.1	88.6
	43.4	67.0	105.7	95.7
	43.4	221.0	269.6	102.0

Average %Recovery: 96.4%

[a] $\%\text{Recovery} = \dfrac{\text{Myoglobin Observed (ng/mL)}}{\text{Endogenous Myoglobin (ng/mL)} + \text{Myoglobin Added (ng/mL)}} \times 100$

DILUTION LINEARITY

Dilution linearity was evaluated by serial dilution of three human serum specimens of known myoglobin concentrations. All specimens were diluted with AxSYM Solution 4 (Line Diluent).

Specimen	Dilution Factor	Expected (ng/mL)	Observed (ng/mL)	%Recovery
A	Undiluted	–	767.7	–
	2	383.9	413.3	107.7
	4	191.9	224.6	117.0
	8	96.0	102.3	106.6
	16	48.0	55.3	115.2
	32	24.0	27.1	112.9
B	Undiluted	–	748.5	–
	2	374.3	375.6	100.3
	4	187.1	179.6	96.0
	8	93.6	93.0	99.4
	16	46.8	47.2	100.9
	32	23.4	25.4	108.5
C	Undiluted	–	–	–
	2	–	982.9	–
	4	491.5	487.7	99.2
	8	245.7	250.0	101.8
	16	122.9	135.4	110.2
	32	61.4	68.0	110.7
	64	30.7	31.7	103.3

$$\%\text{Recovery} = \frac{\text{Observed (ng/mL)}}{\text{Expected (ng/mL)}} \times 100$$

SENSITIVITY

The sensitivity of the AxSYM Myoglobin assay was determined to be <1.0 ng/mL at the 95% confidence interval (n=24). Sensitivity is defined as the concentration at two standard deviations from the mean of the AxSYM Myoglobin Calibrator A rates (in replicates of 10) and represents the lowest measurable concentration of myoglobin that can be distinguished from zero.

SPECIFICITY

The specificity of the AxSYM Myoglobin assay was determined by evaluating cross-reactivity with hemoglobin. Hemoglobin was added to a human serum-based sample at a concentration of 1000 mg/dL and tested using the AxSYM Myoglobin assay. The percent cross-reactivity with hemoglobin was undetectable.

INTERFERENCE

Potential interference from abnormal levels of bilirubin, red blood cells, cholesterol, triglycerides, and total protein was evaluated in the AxSYM Myoglobin assay. The AxSYM Myoglobin assay demonstrated <10% interference in the presence of each of the potentially interfering substances listed below:

Interfering Substance	Concentration
Bilirubin	20 mg/dL
Red Blood Cells	0.4% (v/v)
Cholesterol	500 mg/dL
Triglycerides	2000 mg/dL
Total Protein	3 and 10 g/dL

ACCURACY BY CORRELATION

The AxSYM Myoglobin assay was compared to a commercially available diagnostic kit, Method B Myoglobin. The results of the least squares linear regression analysis are shown below.

Abbott AxSYM Myoglobin vs. Method B Myoglobin

Range (ng/mL)	Number of Observations	Intercept	Slope	Correlation Coefficient
0 - 1,000	383	1.0	1.09	0.981
0 - 10,000	424	10.6	1.02	0.993

For serum and plasma specimens tested between 0 and 1,000 ng/mL, values ranged from 8.6 to 949.3 ng/mL with the AxSYM Myoglobin assay and 8.5 to 844.1 ng/mL with the Method B Myoglobin assay.

For serum and plasma specimens tested between 0 and 10,000 ng/mL, values ranged from 8.6 to 7922.9 ng/mL with the AxSYM Myoglobin assay and 8.5 to 8258.0 ng/mL with the Method B Myoglobin assay.

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