Digitoxin

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.

Key to symbols used

- **REF**: List Number
- **IVD**: In Vitro Diagnostic Medical Device
- **LOT**: Lot Number
- **CAUTION**: Consult accompanying documents
- **i**: Consult instructions for use
- **30°C**: Store at 15-30°C
- **5°C**: Store at 2-8°C
- **EC/REP**: Authorized Representative
- **Expiration Date**
- **CONTROL L**: Control Low, Medium, High (L, M, H)
- **SAMPLE CUPS**: Sample Cups
- **REAGENT PACK**: Reagent Pack
- **STANDARD CALL A**: Standard Calibrator (A - F)
- **REACTION VESSELS**: Reaction Vessels
- **CENTRIFUGE TUBES**: Centrifuge Tubes
- **Manufacturer**: Abbott Laboratories Diagnostics Division
  Abbott Park, IL 60064 USA

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

Read Highlighted Changes
Revised January, 2008
NAME

Digitoxin

INTENDED USE

The AxSYM Digitoxin assay is a reagent system for the quantitative measurement of digitoxin, a cardiovascular drug, in serum or plasma. The measurements obtained are used in the treatment of digitoxin overdose and in monitoring levels of digitoxin to ensure appropriate therapy.

SUMMARY AND EXPLANATION OF TEST

The AxSYM Digitoxin assay utilizes Fluorescence Polarization Immunoassay (FPIA) technology. Refer to the AxSYM System Operations Manual, Section 3, under Principles of Operation for a discussion of this technology.

Digitoxin is a potent cardiac glycoside widely prescribed for the treatment of patients suffering from congestive heart failure, as well as some types of cardiac arrhythmias. Digitoxin intoxication is a common and serious problem in the clinic setting. This is, in part, a result of the fact that cardiac glycosides have a low therapeutic ratio (a very small difference between therapeutic and tissue toxic levels). Coupled with the narrow therapeutic range is a marked patient variability in response to the same dosage of drug, often resulting in unpredictable serum drug levels. Intoxication symptoms are often indistinguishable from the original condition for which the drug was prescribed. It may not be immediately apparent whether the patient has been under or overdosed. Monitoring serum digitoxin levels combined with other clinical data can immediately apparent whether the patient has been under or overdosed. Monitoring serum digitoxin levels combined with other clinical data can provide the physician with useful information to aid in adjusting patient dosage, achieving optimal therapeutic effect while avoiding useless subtherapeutic or harmful toxic dosage levels.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The AxSYM Digitoxin assay is based on Fluorescence Polarization Immunoassay (FPIA) technology. The AxSYM Digitoxin Reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

Solution 4 and pretreated sample required for one test are pipetted by the sampling probe into the Reaction Vessel (RV) cuvette.

Two aliquots of pretreated sample are pipetted into the sample well of the RV.

Aliquots of the Digitoxin Antiserum and Pretreatment Solution are pipetted to RV wells 1 and 2 respectively.

An aliquot of the Pretreatment Solution from RV well 2 is dispensed to the RV cuvette.

An aliquot of the Digitoxin Fluorescein Tracer is dispensed to RV well 3.

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center with the processing probe.

PROCESSING CENTER

Solution 4 and an aliquot of the sample from the RV sample well are dispensed to the RV cuvette.

Solution 4 and an aliquot of the Digitoxin Antiserum from RV well 1 are dispensed to the RV cuvette.

Solution 4 and an aliquot of the Digitoxin Fluorescein Tracer from RV well 3 are dispensed to the RV cuvette.

Solution 4 and another aliquot of the Pretreatment Solution from RV well 2 are dispensed to the RV cuvette.

Digitoxin from the sample and the Digitoxin Fluorescein Tracer compete for binding sites on the antibody molecules.

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 Digitoxin Reagent Pack (3B37-20)*

1 Bottle (9.3 mL) <1% Digitoxin Antiserum (Rabbit, Polyclonal) in phosphate buffer with protein stabilizers. Preservative: Sodium Azide. (Reagent Bottle 1)

1 Bottle (5.0 mL) Pretreatment Solution. Surfactant in TRIS Buffer. Preservative: Sodium Azide. (Reagent Bottle 2)

1 Bottle (22.9 mL) <0.01% Digitoxin Fluorescein Tracer in TRIS Buffer with protein stabilizers. Preservative: Sodium Azide. (Reagent Bottle 3)

* 3B37-99 includes an AxSYM Digitoxin Reagent Pack (100 tests), Reaction Vessels (100 each) and Centrifuge Tubes (100 each).

PRECIPITATION REAGENT REQUIRED BUT NOT SUPPLIED:

- Methanol (Reagent grade or equivalent)

CALIBRATORS

AxSYM Digitoxin Standard Calibrators (3B37-01)

6 Bottles (5.75 mL A, 5.0 mL each B-F) of AxSYM Digitoxin Standard Calibrators contain accurately measured amounts of digitoxin prepared in processed human plasma to yield the following concentrations:

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Digitoxin Concentration</th>
<th>Digitoxin Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/mL &amp; μg/L)</td>
<td>(nmol/L)</td>
</tr>
<tr>
<td>STANDARD CAL A</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>STANDARD CAL B</td>
<td>5.0</td>
<td>6.55</td>
</tr>
<tr>
<td>STANDARD CAL C</td>
<td>10.0</td>
<td>13.10</td>
</tr>
<tr>
<td>STANDARD CAL D</td>
<td>20.0</td>
<td>26.20</td>
</tr>
<tr>
<td>STANDARD CAL E</td>
<td>40.0</td>
<td>52.40</td>
</tr>
<tr>
<td>STANDARD CAL F</td>
<td>80.0</td>
<td>104.80</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide.

Abbott manufactures internal reference standards using Digitoxin (USP Reference Standard). Digitoxin calibrators are manufactured gravimetrically and tested against these internal reference standards.

CONTROLS

AxSYM Digitoxin Controls (3B37-10)

3 Bottles (5.0 mL each) of AxSYM Digitoxin Controls contain digitoxin prepared in processed human plasma to yield the following concentration ranges:

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Concentration</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/mL &amp; μg/L)</td>
<td>(nmol/L)</td>
</tr>
<tr>
<td>CONTROL L</td>
<td>7.5</td>
<td>9.83</td>
</tr>
<tr>
<td>CONTROL M</td>
<td>15.0</td>
<td>19.65</td>
</tr>
<tr>
<td>CONTROL H</td>
<td>35.0</td>
<td>45.85</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide.

OTHER REAGENTS

AxSYM Probe Cleaning Solution (9A35-05)

PROBE CLEANING SOLUTION

2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammoniumhydroxide (TEAH).

Solution 4 (Line Diluent) (8A46)

SOLUTION 4 LINE DILUENT

1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1M Phosphate Buffer. Preservatives: Sodium Azide and Antimicrobial Agent.

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use.

SAFETY PRECAUTIONS

- CAUTION: This product contains human sourced 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, 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. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

- The human plasma used in the AxSYM Digitoxin Calibrators and Controls is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/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.
• The Pretreatment Solution is classified per applicable European Community (EC) Directives. The following are the appropriate Risk (R) and Safety (S) phrases.
  R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
  S35 This material and its container must be disposed of in a safe way.
  S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

• 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 kits beyond the expiration date or a maximum of 224 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

\[ \text{Storage Temperature: } 1-8°C \]

The AxSYM Digitoxin Reagent Pack, Calibrators and Controls must be stored at 2-8°C. The AxSYM Digitoxin Reagent Pack, Calibrators and Controls may be used immediately after removal from the refrigerator. Calibrators and Controls should be returned to 2-8°C storage immediately after use. Do not freeze AxSYM Digitoxin Reagents.

The AxSYM Digitoxin Reagent Pack may be on-board the AxSYM System for a maximum of 224 cumulative hours; for example, 28 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 Appendices, for further information on tracking on-board time.

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

\[ \text{Storage Temp: } 15-30°C \]

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

INSTRUMENT PROCEDURE

ASSAY FILE INSTALLATION

The AxSYM Digitoxin Assay File must be installed on the AxSYM System from one of the following software disks, prior to performing Digitoxin assay:
• 2C4S-02, or higher (112 hours on-board Stability)
• 3D5S-01, or higher (224 hours on-board Stability)

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

AxSYM DIGITOXIN ASSAY PARAMETERS

The default values for the assay parameters used for the AxSYM Digitoxin 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.

<table>
<thead>
<tr>
<th>Assay Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Long Assay Name (English): Digitoxin</td>
<td></td>
</tr>
<tr>
<td>2 Abbrev Assay Name (English): Digitoxin</td>
<td></td>
</tr>
<tr>
<td>3 Assay Number:</td>
<td>617</td>
</tr>
<tr>
<td>4 Assay Version:</td>
<td>*</td>
</tr>
<tr>
<td>5 Calibration Version:</td>
<td>*</td>
</tr>
<tr>
<td>6 Assay File Revision:</td>
<td>*</td>
</tr>
<tr>
<td>7 Assay Enabled &gt; ON</td>
<td></td>
</tr>
<tr>
<td>8 Assay Type: (PIA)</td>
<td></td>
</tr>
<tr>
<td>9 Standard Cal Reps &gt; 2</td>
<td></td>
</tr>
<tr>
<td>10 Cal A Concentration:</td>
<td>0.00</td>
</tr>
<tr>
<td>11 Cal B Concentration:</td>
<td>5.00</td>
</tr>
<tr>
<td>12 Cal C Concentration:</td>
<td>10.00</td>
</tr>
<tr>
<td>13 Cal D Concentration:</td>
<td>20.00</td>
</tr>
<tr>
<td>14 Cal E Concentration:</td>
<td>40.00</td>
</tr>
<tr>
<td>15 Cal F Concentration:</td>
<td>80.00</td>
</tr>
<tr>
<td>16 Default Dilution Protocol &gt; UNDILUTED</td>
<td></td>
</tr>
<tr>
<td>17 Default Calibration Method:</td>
<td>Standard Calibration</td>
</tr>
<tr>
<td>18 Selected Result Concentration Units &gt; ng/mL</td>
<td></td>
</tr>
<tr>
<td>19 Selected Result Decimal Places &gt; 2</td>
<td></td>
</tr>
<tr>
<td>20 Blank I-Max background intensity:</td>
<td>*</td>
</tr>
<tr>
<td>21 Min Tracer-Min net intensity:</td>
<td>*</td>
</tr>
<tr>
<td>22 Low Limit-Normal/Therapeutic Range lower limit &gt; 0.00</td>
<td></td>
</tr>
<tr>
<td>23 Hi Limit-Normal/Therapeutic Range upper limit &gt; 0.00</td>
<td></td>
</tr>
<tr>
<td>24 Low Extreme Value &gt; 0.00</td>
<td></td>
</tr>
<tr>
<td>25 High Extreme Value &gt; 0.00</td>
<td></td>
</tr>
<tr>
<td>26 Low Range Undiluted:</td>
<td>*</td>
</tr>
<tr>
<td>27 High Range Undiluted:</td>
<td>*</td>
</tr>
</tbody>
</table>

NOTE: Assay Parameter #45 can be edited to the alternate result unit of µg/L or nmol/L.

Values associated with the Low and High Extreme flags, Assay Parameters #75 and #76, are assay specific and should not be edited.

We recommend that you set General Configuration Parameter, Release Mode, 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 General Configuration Parameter, Release Mode, is configured to the “Automatic” release mode, ensure that all flagged results are reviewed prior to reporting assay results.

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

• Serum or plasma (collected in sodium heparin or potassium oxalate collection tubes) may be used in the AxSYM Digitoxin assay. Follow the manufacturer’s processing instructions for serum collection tubes.

  NOTE: Do not use plasma containing citrate as an anticoagulant; this may give falsely elevated values for digitoxin.

  Use of EDTA as an anticoagulant is associated with a negative bias in results. EDTA is not recommended for use as an anticoagulant.

• Ensure that complete clot formation has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

  Specimens containing particulate matter or red blood cells may give inconsistent results and should be centrifuged before testing (recommended 8,000-10,000 RCF* x 10 minutes).

  Samples may be stored for up to 24 hours at 2-8°C prior to being tested. If testing will be delayed more than 24 hours, the serum or plasma should be separated from the clot or red blood cells and stored frozen at -10°C or colder for up to 168 hours.

  A pretreatment step must be performed on each digitoxin sample (calibrators, controls, and patient samples) before testing.

  The AxSYM System does not provide the capability of verifying sample type. It is the responsibility of the operator to verify the correct sample type is used in the Digitoxin assay.

  To minimize the effect of sample evaporation, all samples must be run as STAT tests.

  Inspect all extracted samples for bubbles. Remove bubbles prior to analysis.

  When shipped, samples must be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical samples and etiologic agents.

*Relative Centrifugal Force.
SAMPLE PRETREATMENT

- A pretreatment step must be performed on each digitoxin sample (calibrators, controls and patient samples) before testing. The pretreatment step minimizes interference from endogenous protein-bound fluorescent compounds due to the sample volume required for the AxSYM Digitoxin assay. The pretreatment consists of the addition of methanol to the serum or plasma sample in order to precipitate protein, followed by centrifugation to obtain a clear supernatant. The AxSYM Digitoxin assay is then performed on the sample supernatant.

1. Digitoxin calibrators, controls and patient samples must be prepared by the same procedure. Use the Precision Dispenser for accurate dispensing of the methanol.
2. Number a centrifuge tube for each sample to be tested and place in a suitable rack. A maximum of 20 to 24 samples (dependent on centrifuge used) can be centrifuged at one time.
3. Accurately pipet 200 μL of the serum or plasma sample to be assayed into an appropriately labeled centrifuge tube.
4. Set the Precision Dispenser to dispense 300 μL and fill it with methanol. Purge the syringe of air bubbles by dispensing into a suitable waste container. Dispense 300 μL of methanol twice (total of 600 μL) into each centrifuge tube.
5. After dispensing the methanol, cap each centrifuge tube as soon as possible to prevent evaporation of methanol. Mix each on the vortex for 5-10 seconds to ensure thorough mixing.
6. Place the tubes into the centrifuge head.
7. If fewer than the maximum number of samples are loaded into the centrifuge, distribute the tubes evenly so that the head is balanced. Imbalance during centrifugation will result in a poor pellet and potential damage to the centrifuge motor.
8. Centrifuge the samples for at least 3 minutes at 9,500 x g, or until a clear supernatant and a hard, compact pellet of denatured protein is obtained. If centrifugation time is not sufficient, two problems could arise.
   - Too small a volume of supernatant will be available for transfer to the sample cup. A minimum of 429 μL of supernatant is required to perform the AxSYM Digitoxin assay.
   - Floating pieces of unsedimented protein will be transferred to the sample cup. These can clog the probe and cause poor reproducibility in sampling.
9. After centrifugation is complete, verify presence of protein pellet, uncap each tube and immediately decant the supernatant into an appropriately labeled centrifuge tube. The supernatant for at least 3 minutes at 9,500 x g, or until a clear supernatant and a hard, compact pellet of denatured protein is obtained. If centrifugation time is not sufficient, two problems could arise.
   - Too small a volume of supernatant will be available for transfer to the sample cup. A minimum of 429 μL of supernatant is required to perform the AxSYM Digitoxin assay.
   - Floating pieces of unsedimented protein will be transferred to the sample cup. These can clog the probe and cause poor reproducibility in sampling.
   - The assay should be run immediately after decanting supernatants into the sample cups to prevent evaporation of the methanol. Discard any remaining pretreated samples after testing is complete. Do not use remaining pretreated samples for any repeat testing.

Refer to the AxSYM System Operations Manual, Section 5, for a discussion of additional sample handling precautions.

SAMPLE VOLUME

Refer to the SAMPLE PRETREATMENT Section for a description of sample volume requirements and handling.

The AxSYM Digitoxin assay must be requested STAT on the AxSYM System. The pretreated sample volume required to perform a single digitoxin test on the AxSYM System is 429 μL. The sample cup minimum volumes for STAT tests are calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is(are) ordered. DO NOT USE THE AUTO RETEST FEATURE WITH THIS ASSAY.

For calibrator and control requirements for multiple reagent lots, refer to the AxSYM System Operations Manual, Section 5.

AxSYM DIGITOXIN PROCEDURE

Materials Provided

- 3B37-99 AxSYM Digitoxin Reagent Kit, containing:
  - AxSYM Digitoxin REAGENT PACK
  - REACTION VESSELS
  - CENTRIFUGE TUBES

Materials Required But Not Provided

- 3B37-01 AxSYM Digitoxin Standard Calibrators
- 3B37-10 AxSYM Digitoxin Controls
- Methanol (Reagent Grade or equivalent)
- 8A46 SOLUTION X LINE DILUENT
- 9A35-05 AxSYM PROBE CLEANING SOLUTION
- 9A76-01 XSYSTEMS Centrifuge or TDX Centrifuge
- Pipettor and pipette tips
- 9S27-01 Precision Dispenser
- Combi-tips (5.0 mL)

CAUTION:

- 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

To minimize potential for sample evaporation on-board, all digitoxin tests must be requested STAT.

Before running a digitoxin test, update the Order Status Screen. Determine the number of STATs and calibrators pending in the Order Status Screen. Pending STATs, calibrators and desired digitoxin tests must not total more than 60 tests.

Pending STATs + Pending Calibrators + Desired Digitoxin tests ≤ 60

The assay should be run immediately after decanting supernatants into the sample cups. Discard any remaining pretreated samples after testing is complete. A fresh aliquot of pretreated sample should be dispensed to a new cup for all AxSYM Digitoxin tests reordered.

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 bulk solutions and waste levels are acceptable.

The Orderlist Report contains sample placement information and STAT sample volume requirements for all ordered tests. It is recommended that this report be referenced when loading samples into sample segments.

NOTE: The AxSYM Digitoxin assay CANNOT be used with the Host Order Query function on the AxSYM System.

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).
- When only performing FPIA assays, the instrument homes all motors and may display “Error Code 5066 Matrix cell not detected, trap door, processing center”. Select OK to proceed with testing the FPIA assays.
- 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 the AxSYM Digitoxin Reagent Pack is removed from the Sampling Center to maximize the on-board reagent pack use. Store Reagent Pack protected from light at 2-8°C.
QUALITY CONTROL PROCEDURES

CALIBRATION

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

Standard Calibration

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

To ensure sufficient volume to run each calibrator in duplicate, prepare two extractions of each calibrator then combine both extractions into a single sample cup.

Once the AxSYM Digitoxin 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
- Control values are out of their specified 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, Appendices, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

Operator Verification

An acceptable Digitoxin calibration curve should meet the following criteria:

a) Polarization Error (PERR) -3.50 to +3.50 for all calibrators.

b) Root Mean Squared Error (RMSE) less than or equal to 3.00.

c) L, M and H controls are all within the acceptable ranges.

NOTE: PERR’s and RMSE’s 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 Digitoxin assay is a single sample of at least two different digitoxin control levels, which span the medical decision range, 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 this package insert. Refer to the REAGENTS, CONTROLS section of this package insert for AxSYM Digitoxin Control ranges.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When a 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, Subsection: Observed Problems, 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

The AxSYM Digitoxin assay utilizes a Four Parameter Logistic Curve Fit method (4PLC, Y-weighted) to generate a calibration curve. This curve is stored in memory and concentrations of drug in controls and unknown samples are calculated from this curve using polarization values generated.

Flags

Some results may contain information in the Flags field. Samples flagged as low extreme values (LL), Assay parameter #75, must be reviewed prior to reporting assay results. Results at or near the assay sensitivity should be verified prior to reporting drug concentrations.

For a description of the other flags that may appear in this field, refer to the AxSYM System Operations Manual, Section 2.

LIMITATIONS OF THE PROCEDURE

As with all analytics determinations, the digitoxin value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures. Do not use this assay to measure digitoxin in the presence of coadministered digoxin.

Samples for the AxSYM Digitoxin assay may be drawn at least six hours after the last oral dose has been administered, by which time a steady state between serum digitoxin concentration and myocardial digitoxin concentration has been reached.

SAMPLE DILUTION PROCEDURE

CAUTION: Digitoxin samples CANNOT be diluted automatically on the AxSYM.

Manual Dilution Protocol

Patient samples with digitoxin concentrations reported as greater than 80.0 ng/mL may be diluted using a manual dilution of 1:2. Add one part of the patient sample (prior to the precipitation step) to one part of the AxSYM Digitoxin Calibrator A. Repeat the test using this manually diluted sample. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration. Dilutions greater than 1:2 should not be performed.

Expected Values

Numerous studies have shown a relationship between serum levels of digitoxin and its therapeutic effectiveness. Optimum therapeutic effects are usually observed when serum levels are in the range from 10.0 to 30.0 ng/mL. Below 10.0 ng/mL, the patient generally receives little relief from symptoms, and, above 30.0 ng/mL, the patient may begin to experience discomforting toxic symptoms.11-13 These symptoms may include gastrointestinal disturbances such as nausea, vomiting and diarrhea, nervous system disturbances manifested by blurred vision, headache and general weakness, and cardiac arrhythmia and slowing of the pulse.6

It is important to note that distinction between adequate digitalization and toxicity in patients can not be made on the basis of digitoxin concentrations alone. Most studies show a significant overlap between the toxic and non-toxic groups.12 Additional factors to consider when evaluating the correct therapeutic dosage for each patient are age, thyroid condition, acid-base balance, hypoxia, hypokalemia, renal function and other clinical factors.13

Refer to the drug manufacturer’s package insert or the Physicians’ Desk Reference® (PDR) for proper drug dosage and digitoxin measurement sample times.

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

Precision was determined as described in National Committee for Clinical Laboratory Standards (NCCLS) protocol EPS-T214 (including an additional estimate of between day precision) using processed human plasma with 7.5, 15.0, and 35.0 ng/mL of digitoxin added. Results from these studies typically yielded CV’s of less than 13%.

The following are representative results from a pooled variance analysis of data from one reagent lot tested across four instruments.

<table>
<thead>
<tr>
<th>Target value (n=80)</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>15.0</td>
</tr>
<tr>
<td>Mean</td>
<td>7.59</td>
</tr>
<tr>
<td>SD Within Run</td>
<td>0.67</td>
</tr>
<tr>
<td>CV Within Run (%)</td>
<td>8.9</td>
</tr>
<tr>
<td>SD Between Day</td>
<td>0.22</td>
</tr>
<tr>
<td>CV Between Day (%)</td>
<td>2.9</td>
</tr>
<tr>
<td>SD Total</td>
<td>0.72</td>
</tr>
<tr>
<td>CV Total (%)</td>
<td>9.5</td>
</tr>
</tbody>
</table>
ACCURACY BY RECOVERY

Recovery was determined by adding digoxin to human serum and to buffer at concentrations of 8.0, 16.0, 32.0, 40.0, and 72.0 ng/mL. The concentration of digoxin was determined using the AxSYM Digitoxin assay, and the resulting % recovery was calculated according to the following equation:

\[
\% \text{ Recovery} = \left( \frac{\text{serum concentration}}{\text{buffer concentration}} \right) \times 100
\]

Representative data are shown in the following table.

<table>
<thead>
<tr>
<th>Added Concentration (ng/mL)</th>
<th>Concentration in Serum (ng/mL)</th>
<th>Concentration in Buffer (ng/mL)</th>
<th>Percent (%) Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>8.35</td>
<td>8.14</td>
<td>102.15</td>
</tr>
<tr>
<td>16.0</td>
<td>16.49</td>
<td>15.66</td>
<td>104.03</td>
</tr>
<tr>
<td>32.0</td>
<td>33.14</td>
<td>32.58</td>
<td>101.72</td>
</tr>
<tr>
<td>40.0</td>
<td>41.09</td>
<td>40.07</td>
<td>102.56</td>
</tr>
<tr>
<td>72.0</td>
<td>73.05</td>
<td>72.35</td>
<td>96.14</td>
</tr>
</tbody>
</table>

Average Recovery: 101.3 ± 3.0%

SENSITIVITY

The sensitivity of the AxSYM Digitoxin assay was calculated to be 3.0 ng/mL. This sensitivity is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence.

SPECIFICITY

Cross-reactivity was tested for compounds whose chemical structure or concurrent usage could cause potential interference with the AxSYM Digitoxin assay. Digoxin is a rare metabolite of digoxin. Digitoxin samples (7.5 and 35.0 ng/mL) which contained 2 ng/mL digoxin showed <0.3 ng/mL incremental change. Additionally, cross-reactivity of digoxin and digitoxin metabolites were also established by spiking drug-free pooled human serum with the test compounds and then assaying the sample by the Digitoxin assay. The results of cross-reactivity studies are shown in the following table.

<table>
<thead>
<tr>
<th>Tested Compounds</th>
<th>Concentration (ng/mL)</th>
<th>Average % Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digitoxin</td>
<td>30 ng/mL</td>
<td>10</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>25 ng/mL</td>
<td>109.4</td>
</tr>
<tr>
<td>Digitoxigen-mono-digitoxoside</td>
<td>25 ng/mL</td>
<td>151.4</td>
</tr>
<tr>
<td>Digitoxigen-bis-digitoxoside</td>
<td>25 ng/mL</td>
<td>132.5</td>
</tr>
</tbody>
</table>

The results of cross-reactivity studies with the following compounds, steroids and drugs commonly co-administered with digoxin are shown in the following table.

<table>
<thead>
<tr>
<th>Tested Compounds</th>
<th>Concentration (ng/mL)</th>
<th>Average % Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrodigoxin</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Dihydrodigitoxigen</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Dihydrodigoxin</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Dihydrodigitoxigen</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Dihydrodigitoxigen</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Dihydrodigitoxigen</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>1 μg/mL</td>
<td>0.76</td>
</tr>
<tr>
<td>Ouabain</td>
<td>1 μg/mL</td>
<td>0.76</td>
</tr>
<tr>
<td>Progesterone</td>
<td>5 μg/mL</td>
<td>0.37</td>
</tr>
<tr>
<td>Testosterone</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Testosterone</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Testosterone</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Prednisone</td>
<td>25 ng/mL</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = None Detected; Concentration < assay sensitivity (3.0 ng/mL). 

INTERFERENCE

The following compounds, added to human serum, resulted in less than 10% error in determining added drug when assayed with the AxSYM Digitoxin assay.

- Bilirubin
- Hemoglobin
- Triglycerides
- Total Protein

NOTE: Do not use plasma containing citrate as an anticoagulant; this may give falsely elevated values for digoxin. Use of EDTA as an anticoagulant is associated with a negative bias in results.

EDTA is not recommended for use as an anticoagulant. Because digoxin is highly protein bound (greater than or equal to 90%) in serum samples, human serum with protein in concentrations of 3.5 and 11.0 g/dL were spiked with digoxin and tested for recovery. Recovery was greater than 90% at all protein concentrations tested.

ACCURACY BY CORRELATION

The Abbott AxSYM Digitoxin assay was compared to commercially available Fluorescence Polarization Immunoassays. The results of the specimen testing are shown in the following table.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Number of Observations</th>
<th>Intercept</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott AxSYM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digitoxin vs.</td>
<td></td>
<td>517</td>
<td>0.58</td>
<td>0.96</td>
</tr>
<tr>
<td>Abbott TDX/TDXFLx</td>
<td>Digitoxin Assay</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample Range (AxSYM): 3.10 to 51.02 ng/mL.

AxSYM, XSYSTEMS, TDX and TDXFLx are trademarks of Abbott Laboratories.

BIBLIOGRAPHY


RELATED READINGS