Digoxin II

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.

If you receive reagents, calibrators, controls or bulk solutions that are in a condition contrary to the package insert or label recommendation, or that are damaged, contact your area Customer Support.

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Key to symbols used

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

See REAGENTS section for a full explanation of symbols used in reagent component naming.
AxSYM Digoxin II assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative measurement of digoxin, a cardiovascular drug, in serum or plasma. The measurements obtained are used in the treatment of digoxin overdose and in monitoring levels of digoxin to ensure appropriate therapy.

**SUMMARY AND EXPLANATION OF TEST**

Digoxin 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. Digoxin intoxication is a common and serious problem in the clinical 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 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 digoxin 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 or harmful toxic dosage levels.

**BILOGICAL PRINCIPLES OF THE PROCEDURE**

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

**SAMPLING CENTER**

- Sample and all AxSYM Digoxin II Reagents required for one test are pipetted by the sampling probe into various wells of a Reaction Vessel (RV).
- Sample and Anti-digoxin Coated Microparticles are combined in the appropriate well in the RV.

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

**PROCESSING CENTER**

- The digoxin binds to the Anti-digoxin Coated Microparticles forming an "antibody-antigen" complex.
- An aliquot of the reaction mixture containing the "antibody-antigen" complex bound to the microparticles is transferred to the matrix cell. The microparticles bind irreversibly to the antibody-antigen complex bound to the matrix cell.
- The Digoxin: Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds to the available sites on the Anti-digoxin Coated Microparticles forming an "antibody-antigen" 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 is measured by the MEIA optical assembly.

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

**REAGENTS**

**REAGENT PACK, 100 TESTS**

- 1 Bottle (15.0 mL) Digoxin: Alkaline Phosphatase Conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.05 µg/mL. Preservatives: Sodium Azide and Antimicrobial Agents.
- 1 Bottle (7.6 mL) Anti-digoxin (Rabbit) Coated Microparticles in TRIS buffer with protein stabilizers. Minimum concentration: 0.05 µg/mL. Preservative: Sodium Azide.
- 1 Bottle (15.0 mL) Digoxin: Alkaline Phosphatase Conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.0001%. Preservative: Sodium Azide.
- 1 Bottle (27.0 mL) MEIA Buffer containing 0.3M Sodium Chloride in AMP buffer. Preservatives: Sodium Azide and Antimicrobial Agents.
- 1 Bottle (41.9 mL) Digoxin II Probe Wash Solution containing a 2.0M Sodium Chloride solution. Preservative: Sodium Azide.

No. 5B73-20 includes an AxSYM Digoxin II Reagent Pack (100 tests), reaction vessels (100 each) and matrix cells (100 each). No. 5B73-20 includes these items for international shipments.

**CALIBRATORS**

**Digoxin Calibrators (9C14-01)**

- 6 Bottles (6 mL A, 4 mL each B-F) of Digoxin Calibrators contain accurately measured amounts of digoxin prepared in human serum to yield the following concentrations:

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Concentration (ng/mL)</th>
<th>Range (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL A</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>CAL B</td>
<td>0.5</td>
<td>0.64</td>
</tr>
<tr>
<td>CAL C</td>
<td>1.0</td>
<td>1.28</td>
</tr>
<tr>
<td>CAL D</td>
<td>2.0</td>
<td>2.56</td>
</tr>
<tr>
<td>CAL E</td>
<td>3.0</td>
<td>3.84</td>
</tr>
<tr>
<td>CAL F</td>
<td>4.0</td>
<td>5.12</td>
</tr>
</tbody>
</table>

**CONTROLS**

**Digoxin Controls (9C14-10)**

- 3 Bottles (8 mL each) of Digoxin Controls contain digoxin prepared in human serum to yield the following concentration ranges:

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Concentration (ng/mL)</th>
<th>Range (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL L</td>
<td>0.9 - 1.15</td>
<td>0.60 - 1.20</td>
</tr>
<tr>
<td>CONTROL M</td>
<td>1.9 - 2.43</td>
<td>1.50 - 2.30</td>
</tr>
<tr>
<td>CONTROL H</td>
<td>3.2 - 4.10</td>
<td>2.60 - 3.80</td>
</tr>
</tbody>
</table>

**PROBE CLEANING SOLUTION**

- 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 3% Tetraethylammoniumhydroxide (TEAH).

**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)**

- 4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3M Sodium Chloride in TRIS Buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

**SOLUTION 4 (LINE DILUENT)**

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

**WANNINGS 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. 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 serum used in the Digoxin Calibrators and Controls is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2 and anti-HCV.
  - This reagent 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 Digoxin II Reagents are susceptible to bubbles/foaming and require inspection and removal of bubbles before loading. If bubbles are present, refer to the AxSYM System Operations Manual, Sections 2, 5, and Appendices for instructions on removing bubbles from reagent packs.
- Do not use Solution 1 (MUP) beyond the expiration date or a maximum of fourteen 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 to air may compromise performance.
- 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

- The AxSYM Digoxin II Reagent Pack, Digoxin Calibrators and Digoxin Controls must be stored at 2-8°C. The AxSYM Digoxin II Reagent Pack, Digoxin Calibrators and Digoxin 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 Digoxin II Reagents.
- The AxSYM Digoxin II 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. Solution 1 (MUP) must be stored at 2-8°C. Do not freeze MUP. It may be used immediately after removing it from the refrigerator. MUP may be on-board the AxSYM System for a maximum of fourteen days. After fourteen days, it must be discarded.

NOTE: When ordering test(s) for the AxSYM Digoxin II assay, “Digoxin” must be selected from the assay list. *AxSYM Digoxin II Assay Parameters*

The default values for the assay parameters used for the AxSYM Digoxin II 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>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbrev Assay Name</td>
<td>Digoxin</td>
</tr>
<tr>
<td>Assay Name</td>
<td>Digoxin II</td>
</tr>
<tr>
<td>Assay Version</td>
<td>1.1</td>
</tr>
<tr>
<td>Calibration Version</td>
<td>1.1</td>
</tr>
<tr>
<td>Assay File Revision</td>
<td>1.1</td>
</tr>
<tr>
<td>Assay Enabled</td>
<td>ON</td>
</tr>
<tr>
<td>Assay Type</td>
<td>MEIA</td>
</tr>
<tr>
<td>Standard Cal Reps</td>
<td>2</td>
</tr>
<tr>
<td>Cal A Concentration</td>
<td>0.00</td>
</tr>
<tr>
<td>Cal B Concentration</td>
<td>0.50</td>
</tr>
<tr>
<td>Cal C Concentration</td>
<td>1.00</td>
</tr>
<tr>
<td>Cal D Concentration</td>
<td>2.00</td>
</tr>
<tr>
<td>Cal E Concentration</td>
<td>3.00</td>
</tr>
<tr>
<td>Cal F Concentration</td>
<td>4.00</td>
</tr>
<tr>
<td>Default Dilution Protocol</td>
<td>UNDILUTED</td>
</tr>
</tbody>
</table>

NOTE: Parameter #45 can be edited to alternate result unit mmol/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, citrate, EDTA, or oxalate collection tubes) may be used in the AxSYM Digoxin II assay. Follow the manufacturer’s processing instructions for serum or plasma collection tubes.
- 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(s) is/are used in the Digoxin II assay.
- Ensure that complete clot formation has taken place prior to centrifugation. Some samples, especially those from patients receiving anti-coagulant 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.
- Patients receiving potassium canrenoate or certain corticosteroids by intravenous infusion should have blood drawn for digoxin determination prior to the start of the infusion. Refer to SPECIFIC PERFORMANCE CHARACTERISTICS, INTERFERENCE section.
- 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.
- Refer to the AxSYM System Operations Manual, Section 5, for a detailed discussion of on-board sample storage constraints.
- Inspect all 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 hazardous materials.
- Relative Centrifugal Force.

SAMPLE VOLUME

The sample volume required to perform a single undiluted digoxin 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 131 µL. For every additional digoxin test performed (ROUTINE or STAT) from the same sample container, an additional 81 µL of sample is required. The sample cup minimum volumes for both ROUTINE and STAT tests (undiluted or diluted) are calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is/are ordered. When using Host Order Query, the Order screen information and Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5: Ordering Patient Samples, 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 the additional sample listed above.
To obtain the recommended volume requirements for Digoxin Calibrators and Controls, hold the bottles **vertically** and dispense 4 drops of each calibrator or 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 AxSYM Digoxin II reagent lots.

**AxSYM DIGOXIN II PROCEDURE**

**Materials Provided**
- 8B73-66 AxSYM Digoxin II Reagent Kit, containing:
  - AxSYM Digoxin II Reagent Pack
  - 100 **REACTION VESSELS**
  - 100 **MATRIX CELLS**

**Materials Required But Not Provided**
- 9C14-01 Digoxin Calibrators
- 9C14-10 Digoxin Controls
- 8A47-04 Solution 1 (MUP)
- 8A81-04 Solution 3 (Matrix Cell Wash)
- 8A46 Solution 4 (Line Diluent)
- 9A35-05 AxSYM Probe Cleaning Solution
- 8A76-01 **SAMPLE CUPS**
- Pipette and pipette tips (optional) to deliver the volumes specified on the order screen.

**CAUTION:**
- For optimal performance it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 5. 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), matrix cells, bulk solutions and waste levels are acceptable.

**NOTE:** When ordering test(s) for the AxSYM Digoxin II assay, "Digoxin" must be selected from the assay list.

The operator may obtain an Orderlist Report by pressing PRINT. The printout contains sample placement information and minimum **STAT** sample cup requirements for all tests ordered. When using Host Order Query the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5: Ordering Patient Samples, 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 the AxSYM Digoxin II Reagent Pack is removed from the Sampling Center to maximize the on-board reagent pack use. Store Reagent Pack at 2-8°C.

**Manual Dilution Protocol**

A manual dilution can be performed on patient samples (with digoxin concentrations reported as greater than 4.00 ng/mL) by making a dilution of the specimen with the Digoxin Calibrator A (0 ng/mL) before pipetting the sample into the sample cup. The dilution must be performed so that the diluted test results read greater than the assay sensitivity (0.3 ng/mL).

The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

\[
\text{Final Sample Concentration} = \frac{\text{Volume of Sample} \times \text{Manual Dilution Factor}}{\text{Volume of Dilution Reagent} - \text{Volume of Sample}}
\]

**QUALITY CONTROL PROCEDURES**

**CALIBRATION**

- The AxSYM Digoxin II assay must be calibrated using a Standard Calibration (6-point) procedure.
- **Standard Calibration**
  - To perform a Standard Calibration, test the Digoxin Calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of digoxin controls must be tested as a means of evaluating the assay calibration.
  - Once the AxSYM Digoxin II 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
    - 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, Appendix D, 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 Digoxin II assay is a single sample of at least two different digoxin 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 the package insert. Refer to the **REAGENTS, CONTROLS** section of this package insert for Digoxin 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, Alternate Result Unit, for further information. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

**FLUORESCENCE BACKGROUND ACCEPTANCE CRITERIA**

- Quality control of the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64 (Max Intercept-Max MUP Intercep) each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the test 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**

- The AxSYM Digoxin II 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 rate values generated.

**Alternate Result Unit**

- The default result unit for AxSYM Digoxin II is ng/mL. When selecting the alternate result unit, nmol/L, the conversion factor used by the AxSYM System is 1.28.

**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 1.
LIMITATIONS OF THE PROCEDURE
As with all analytic determinations, the digoxin value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Samples for the AxSYM Digoxin II assay should be drawn at least six hours after the last oral dose has been administered, by which time a steady state between serum digoxin concentration and myocardial digoxin concentration has been reached. Samples from patients receiving digoxin will show falsely elevated values for digoxin. Patients on cardiac digitalis therapy or taking digoxin may show depressed digoxin values due to interference.

SAMPLE DILUTION PROCEDURE
CAUTION: Digoxin samples CANNOT be diluted automatically on the AxSYM.

Patient samples containing the aldosterone inhibitors spironolactone and canrenone at high levels of certain corticosteroids may show negative bias when the AxSYM Digoxin II assay is used. (Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS, INTERFERENCE section.)

EXPECTED VALUES
Numerous studies have shown a relationship between serum levels of digoxin and its concentration in myocardial and other tissues. Optimum therapeutic effects are usually observed when serum levels are in the range from 0.8 to 2.0 ng/mL. Below 0.8 ng/mL, the patient generally receives little relief from symptoms, and above 2.0 ng/mL the patient may begin to experience symptomatic toxicity.5,6 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.5

Mean serum concentrations between 2.0-2.7 ng/mL (range up to 4.3 ng/mL) may be associated with toxicity in adults, exhibit no signs of cardiac rhythm disturbances in young children.7 After two years of age, serum values for children more closely approximate those of adults.

It is important to note that distinction between adequate digitalization and toxicity in patients cannot be made on the basis of digoxin concentrations alone. Most studies show a significant overlap between the toxic and nontoxic groups. 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.9

Refer to the manufacturer's package insert or the Physicians' Desk Reference (PDR) for proper drug dosage and digoxin measurement sample times.9

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

Precision was determined as described in National Committee for Clinical Laboratory Standards (NCCLS) protocol EP-T5220; (including an additional estimate of between day precision) using human serum with 0.9, 1.9, and 3.2 ng/mL of digoxin added. Results from these studies typically yielded CV% of less than 10%. The following are representative results from pooled data from three reagent lots tested across five instruments for a total of nine precision studies.

Sensitivity

The sensitivity of the AxSYM Digoxin II assay was calculated to be 0.3 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 Digoxin II assay. In the absence and presence of digoxin, the digoxin metabolites digoxigenin, digoxigenin mono-digoxiside, and digoxigenin bi-digoxiside, were tested at concentrations corresponding to high plasma levels in digoxin patients (0.132, 0.088 and 0.22 ng/mL, respectively). The studies showed digoxin concentrations of 0.5 ng/mL in absence of digoxin and a change of -0.3 ng/mL in presence of 2.2 ng/mL digoxin. Digitoxin, tested at 25 ng/mL, resulted in a concentration of approximately 0.73 ng/mL in the AxSYM Digoxin II assay. Digitoxin cross-reactivity was also tested in the presence of approximately 2.2 ng/mL digoxin and showed an apparent change in digoxin concentration of approximately -0.82 ng/mL.

INTERFERENCE

Spironolactone and canrenone can interfere with some digoxin assays.22 Reports by Steimer et al.22,23 have shown a negative bias in the determination of digoxin when aldosterone inhibitors spironolactone or canrenone are present in serum. Other aldosterone inhibitors may also potentially result in negative interference in the AxSYM Digoxin II assay. Additionally, corticosteroids given in high doses (e.g., by intravenous administration) may result in negative interference in the AxSYM Digoxin II assay.23

The following representative data was obtained during interference studies conducted at Abbott Laboratories. Serum samples containing approximately 3.30 ng/mL digoxin were spiked with aldosterone inhibitors or other steroid drugs to obtain concentrations shown in the table below. Concentrations of these cross-reacting compounds tested are an estimate of the maximum blood concentrations that could be present in the clinical setting. Serum concentrations of spironolactone and canrenone vary based on route and frequency of administration, measurement methods as well as overall patient metabolic functioning. The test concentrations for spironolactone and canrenone shown in the table below are consistent with reported peak serum concentration values for oral doses of spironolactone of 100 to 200 mg per day.24,26

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration tested (ng/mL)</th>
<th>Percent Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spironolactone</td>
<td>509</td>
<td>-33%</td>
</tr>
<tr>
<td>Canrenone</td>
<td>582</td>
<td>-41%</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>5000</td>
<td>-38%</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>5000</td>
<td>-7%</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>4900</td>
<td>-27%</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4800</td>
<td>-15%</td>
</tr>
<tr>
<td>Progesterone</td>
<td>500</td>
<td>-33%</td>
</tr>
</tbody>
</table>

The MEIA digoxin assays were not tested at canrenone concentration levels higher than 582 ng/mL as they showed a high negative interference at this concentration.

As demonstrated in the table above, high concentrations of hydrocortisone23, prednisolone and dexamethasone may produce significant negative interference. Internal studies have also shown that progesterone, a steroid elevated in late third trimester pregnancy and in newborn infants, causes negative interference in the AxSYM Digoxin II assay.

The sera from patients in specific patient populations (i.e. patients with renal and/or hepatic failure, newborn infants and pregnant women) have been reported to contain an unidentified component that gives positive results for digoxin with a number of immunoassays.24,25 This component has been called digoxin-like immunoreactive factor (DLIF) or substance (DLIS). The presence of DLIF in a sample can result in falsely elevated digoxin assay results. The amount of DLIF in these patient samples is extremely variable, but in some cases these levels have been shown to approach concentrations that are in the therapeutic range of digoxin.14,16

DIGIBIND and DIGIFAB are digoxin-binding immunoglobulin fragments indicated for the treatment of potentially life-threatening digoxin intoxication.19-26 Following administration of DIGIBIND or DIGIFAB to a patient, digoxin immunoassay (MEIA) results will depend on the fraction of digoxin bound to the antibody.20 When the dose of DIGIBIND or DIGIFAB used is high and calculated to bind the entire digoxin body load, resulting digoxin concentrations obtained will be low and approach assay sensitivity (0.3 ng/mL).21 When the dose of digoxin-binding fragment is calculated to bind only a fraction of the digoxin in the patient or when digoxin administration has been reinitiated, digoxin concentrations obtained using the AxSYM assay will be higher.

ACCURACY BY RECOVERY

Recovery was determined by adding digoxin to human serum and to buffer at concentrations of 0.75, 1.50, 2.25, 3.00, and 3.75 ng/mL. The concentration of digoxin was determined using the AxSYM Digoxin II assay, and the resulting % recovery was calculated according to the following equation:

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

The sera from patients in specific patient populations (i.e. patients with renal and/or hepatic failure, newborn infants and pregnant women) have been reported to contain an unidentified component that gives positive results for digoxin with a number of immunoassays.24,25 This component has been called digoxin-like immunoreactive factor (DLIF) or substance (DLIS). The presence of DLIF in a sample can result in falsely elevated digoxin assay results. The amount of DLIF in these patient samples is extremely variable, but in some cases these levels have been shown to approach concentrations that are in the therapeutic range of digoxin.14,16

DIGIBIND and DIGIFAB are digoxin-binding immunoglobulin fragments indicated for the treatment of potentially life-threatening digoxin intoxication.19-26 Following administration of DIGIBIND or DIGIFAB to a patient, digoxin immunoassay (MEIA) results will depend on the fraction of digoxin bound to the Fab fragment.

Following administration of DIGIBIND to a patient, the AxSYM Digoxin II assay will primarily measure the free digoxin, the fraction not bound to the antibody.20 When the dose of DIGIBIND or DIGIFAB used is high and calculated to bind the entire digoxin body load, resulting digoxin concentrations obtained will be low and approach assay sensitivity (0.3 ng/mL).21 When the dose of digoxin-binding fragment is calculated to bind only a fraction of the digoxin in the patient or when digoxin administration has been reinitiated, digoxin concentrations obtained using the AxSYM assay will be higher.

Sensitivity

The sensitivity of the AxSYM Digoxin II assay was calculated to be 0.3 ng/mL. This sensitivity is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence.
The following compounds, when tested with the AxSYM Digoxin II assay at the concentrations indicated, resulted in less than 10% error in detecting digoxin.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>750 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2500 mg/dL</td>
</tr>
<tr>
<td>Protein</td>
<td>3.0 - 12.0 g/dL</td>
</tr>
</tbody>
</table>

**Digoxin II vs. Abbott IMx**

<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>Digoxin II</td>
<td>500</td>
<td>0.08</td>
<td>0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>Digoxin*</td>
<td>501</td>
<td>0.20</td>
<td>0.90</td>
<td>0.93</td>
</tr>
<tr>
<td>Abbott TDx</td>
<td>501</td>
<td>0.08</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>TDX/FLx Digoxin II</td>
<td>112</td>
<td>-0.08</td>
<td>1.07</td>
<td>0.96</td>
</tr>
<tr>
<td>aca Digoxin**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sample Range (AxSYM Digoxin II): 0.31 - 3.88 ng/mL.

**Sample Range (AxSYM Digoxin II): 0.33 - 3.37 ng/mL.

**BIBLIOGRAPHY**

16. Hicks JM, Brett EM. Falsely increased digoxin concentrations in samples from neonates and infants. Ther Drug Monit 1984; 6: 481-4.
19. DIGIBIND (Digoxin Immune Fab (Ovine), GlaxoSmithKline, Inc.) product labeling, August 2001.

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