Digoxin

Ref: 11820796 322

System: Elecsys 2010
MODULAR ANALYTICS E170
cobas e 411
cobas e 601
cobas e 602

English

Intended use

Immunocassay for the in vitro quantitative determination of digoxin in human serum and plasma. Measurements are used in the diagnosis and treatment of digoxin overdose and in monitoring levels of digoxin to ensure proper therapy.

The electrochemiluminescence immunocassay “ECLIA” is intended for use on Elecsys and cobas e immunocassay analyzers.

Summary

Digoxin is widely prescribed for the treatment of congestive heart failure and various disturbances of cardiac rhythm. Therapeutic use of digoxin improves the strength of myocardial contraction and results in the beneficial effects of increased cardiac output, decreased heart size, decreased venous pressure, and decreased blood volume. Digoxin therapy also results in stabilized and slowed ventricular pulse rate. 1

Although the availability of crystalline digoxin has permitted the standardization of drug dosage, therapeutic administration inadvertently, yet frequently, results in toxicity. Importantly, symptoms of digoxin toxicity often mimic the cardiac arrhythmias for which the drug was originally prescribed. Digoxin concentrations of 0.9-2.0 ng/mL in serum or plasma are normally considered to be therapeutic. 2

Symptoms of human toxicity generally only appear at concentrations above 2.0 ng/mL; however, in some cases these symptoms are observed at concentrations as low as 1.4 ng/mL. 3 Based on the “ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008” a therapeutic concentration range for digoxin of 0.6-1.2 ng/mL is recommended. 4 Increased risk of mortality was observed for digoxin concentration of 1.2 ng/mL and higher. 5

Toxicity of digoxin may reflect several factors:

1. The drug has a low therapeutic ratio (i.e. a very small difference exists between therapeutic and toxic tissue levels);
2. Individuals vary in their response to digoxin;
3. Absorption of various tablet forms of digoxin may vary over a two-fold range; 6,7
4. Susceptibility to digitalis toxicity apparently increases with age.

In combination with other clinical data, monitoring serum or plasma levels may provide the physician with useful information to aid in adjusting patient dosage, and achieving optimal therapeutic effect, while avoiding both subtherapeutic and harmful drug levels. 8

The Elecsys Digoxin assay employs a competitive test principle using a monoclonal antibody specifically directed against digoxin. Digoxin in the sample competes with the added digoxin derivative labeled with biotin for the binding sites on the ruthenylated antibody-complex 9.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Reagents - working solutions

The reagent pack contains an antibody, ruthenium-labeled antibody, ruthenylated digoxigenin, biotinylated digoxin, ruthenium-phosphate buffer, and preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid contact with mucous membranes and eyes.

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in from the respective reagent barcode.

Storage and stability

Store at 2-8 °C.
Do not freeze.
Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

- unopened at 2-8 °C: up to the stated expiration date
- after opening at 2-8 °C: 12 weeks
- on the analyzers: 8 weeks

Specimen collection and preparation

Samples for digoxin analyses should preferably be collected 6-8 hours after administration of the drug. 9

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes. Some tubes containing separating gel are not suitable for use in therapeutic drug monitoring; note the data provided by the manufacturer.

Stable for 2 days at 2-8 °C, 6 months at -20 °C. Freeze only once. 7,8

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing. i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could

2014-07, V 19.0 English
Digoxin

Digoxin

affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Heat-inactivated serum can be used. Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement. Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided
See “Reagents – working solutions” section for reagents.

Materials required (but not provided)
- 11820907322, Digoxin CalSet, 4 x 1.5 mL
- 04917049190, PreciControl Cardiac II, for 2 x 2 mL each of PreciControl Cardiac II 1 and 2
- 11732277122, Diluent Universal, 2 x 16 mL sample diluent or 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer

Accessories for Elecsys 2010 and cobas e analyzer
- 11662988122, ProCell, 6 x 380 mL system buffer
- 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- 11933159001, Adapter for SysClean
- 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:
- 04880340190, ProCell M, 2 x 2 L system buffer
- 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- 03023150001, WasteLiner, waste bags
- 03027651001, SysClean Adapter M

Accessories for all analyzers:
- 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary. Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers. Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration
Traceability: This method has been standardized by weighing United States Pharmacopoeia (USP) digoxin reference material into analyte free human serum. Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control
For quality control, use PreciControl Cardiac II.
In addition, other suitable control material can be used. Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation
The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L or ng/mL).

Conversion factors:
- nmol/L x 0.78 = ng/mL
- ng/mL x 1.28 = nmol/L

Limitations - interference
The assay is unaffected by icterus (bilirubin < 1112 μmol/L or < 65 mg/dL), hemolysis (Hb < 0.821 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and bilirubin (< 408 mmol/L or < 100 mg/dL).

Criterior: Recovery within ± 0.15 ng/mL for digoxin concentrations < 1.5 ng/mL (< 1.92 nmol/L) or ± 10 % for concentrations > 1.5 ng/mL (> 1.92 nmol/L).

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
No interference was observed from hematoidin factors up to a concentration of 1630 IU/mL.
In vitro tests were performed on a panel of commonly used pharmaceuticals. While 69 of these showed no interference with the assay, uzara, nabumeton, hydrocortisone, pentoxifylline and canrenone were identified to cause falsely elevated digoxin values at concentrations of the recommended daily dose.
Spironolactone causes elevated digoxin results above (drug) levels of 10000 ng/mL. Canrenone causes elevated digoxin results above (drug) levels of 50000 ng/mL.

Digoxin-like immunoreactive substances (DLS) have been identified in blood from patients in renal failure, liver failure, and pregnant women in their third trimester. Studies have shown that the presence of DLS in a sample can result in a false elevation of digoxin when assayed by commercially available immunoassays.121

The manufacturer of Digoxin Immune FAB (antibody fragment therapy) has stated that no immunoassay technique is suitable for quantitating digoxin in serum from patients undergoing this treatment.13

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.
Digoxin

Limits and ranges

Measuring range

0.15-5.0 ng/mL or 0.19-6.4 nmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.15 ng/mL or < 0.19 nmol/L. Values above the measuring range are reported as > 5.0 ng/mL or > 6.4 nmol/L (or up to 10.0 ng/mL or 12.8 nmol/L for 2-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.15 ng/mL (0.19 nmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with digoxin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or cobas e analyzers or manually). The concentration of the diluted sample must be > 2.5 ng/mL or > 3.2 nmol/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and cobas e software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The usual therapeutic range for digoxin is 0.9-2.0 ng/mL (1.2-2.6 nmol/L). Concentrations above 2.0 ng/mL (2.6 nmol/L) are generally considered toxic. Some overlap of toxic and non-toxic values has been reported.

Therefore, clinical diagnosis should be based on clinical and laboratory data. Each laboratory should establish an acceptable reporting format and identify procedures for the reporting of abnormal results.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EPS-42) of the CLSI (Clinical and Laboratory Standards Institute). 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

<table>
<thead>
<tr>
<th>Elecsys 2010 and cobas e 411 analyzers</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>nmol/L</td>
<td>ng/mL</td>
<td>ng/mL</td>
</tr>
<tr>
<td>HS\textsuperscript{a}</td>
<td>0.68</td>
<td>0.53</td>
</tr>
<tr>
<td>HS 2</td>
<td>1.91</td>
<td>1.49</td>
</tr>
<tr>
<td>HS 3</td>
<td>4.10</td>
<td>3.20</td>
</tr>
<tr>
<td>HS 4</td>
<td>5.90</td>
<td>4.61</td>
</tr>
<tr>
<td>PC\textsuperscript{b} CARD1</td>
<td>1.48</td>
<td>1.16</td>
</tr>
<tr>
<td>PC CARD2</td>
<td>3.64</td>
<td>2.84</td>
</tr>
</tbody>
</table>

\( a \) HS = human serum
\( b \) PC = PreciControl

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/L</td>
<td>ng/mL</td>
<td>ng/mL</td>
<td>%</td>
<td>nmol/L</td>
<td>ng/mL</td>
<td>%</td>
</tr>
<tr>
<td>HS 1</td>
<td>0.65</td>
<td>0.50</td>
<td>0.04</td>
<td>0.03</td>
<td>5.6</td>
<td>0.06</td>
</tr>
<tr>
<td>HS 2</td>
<td>1.84</td>
<td>1.44</td>
<td>0.05</td>
<td>0.04</td>
<td>3.0</td>
<td>0.10</td>
</tr>
<tr>
<td>HS 3</td>
<td>4.05</td>
<td>3.16</td>
<td>0.10</td>
<td>0.08</td>
<td>2.5</td>
<td>0.15</td>
</tr>
<tr>
<td>HS 4</td>
<td>5.70</td>
<td>4.45</td>
<td>0.14</td>
<td>0.11</td>
<td>2.5</td>
<td>0.18</td>
</tr>
<tr>
<td>PC CARD1</td>
<td>1.52</td>
<td>1.19</td>
<td>0.05</td>
<td>0.04</td>
<td>3.0</td>
<td>0.09</td>
</tr>
<tr>
<td>PC CARD2</td>
<td>3.73</td>
<td>2.91</td>
<td>0.09</td>
<td>0.07</td>
<td>2.5</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Method comparison

A comparison of the Elecsys Digoxin assay - standardized against USP reference material - (y) with the Elecsys Digoxin assay - previous test version - (x) using clinical samples gave the following correlations (ng/mL):

- Number of samples measured: 81
- Passing/Bablok\textsuperscript{15} Linear regression
  \[ y = 1.06x - 0.06 \]
  \[ \tau = 0.999 \quad r = 1.000 \]

The sample concentrations were between approximately 0 and 3.2 ng/mL (approximately 0 and 4.10 nmol/L).

Analytical specificity

For the substances used, the following cross-reactivities were found:

<table>
<thead>
<tr>
<th>Substances</th>
<th>Concentration tested ng/mL</th>
<th>Cross-reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acetyldigoxin</td>
<td>5</td>
<td>69.8</td>
</tr>
<tr>
<td>β-acetyldigoxin</td>
<td>5</td>
<td>81.4</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>500</td>
<td>1.13</td>
</tr>
<tr>
<td>Digitoxigenin</td>
<td>2000</td>
<td>0.18</td>
</tr>
<tr>
<td>Digoxigenin</td>
<td>6</td>
<td>14.3</td>
</tr>
<tr>
<td>Digoxigenin-bis-digitoxide</td>
<td>5</td>
<td>74.6</td>
</tr>
<tr>
<td>Digoxigenin-mono-digitoxide</td>
<td>5</td>
<td>55.3</td>
</tr>
<tr>
<td>Dihydrodigoxin</td>
<td>2000</td>
<td>0.14</td>
</tr>
<tr>
<td>β-methyldigoxin</td>
<td>5</td>
<td>91.2</td>
</tr>
<tr>
<td>Deslanoside</td>
<td>10</td>
<td>51.5</td>
</tr>
<tr>
<td>Lanatoside</td>
<td>5</td>
<td>55.1</td>
</tr>
<tr>
<td>Gitalin</td>
<td>150</td>
<td>1.41</td>
</tr>
</tbody>
</table>

No significant cross-reactivity (< 0.01 %) was found for the following substances (tested concentration 5000 ng/mL):

- Ouabain, k-strophantidine, cortisol, corticosterone, cortisone, 11-deoxycortic, 21-deoxycortic, prednisolone, prednisone, 6-alpha-methyl-prednisolone, progesterone, 17-hydroxyprogesterone, testosterone, β-estradiol, estrol, d-aldosterone, β-DHEA, DHEA, dexamethasone, betamethasone, triamcinolone, furosemide, theophylline, sulthiam, quinine (free base) and oleandrin.

References

Digoxin


4 Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur Heart J 2008;29:2388-2442.


For further information, please refer to the appropriate operator’s manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integer and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols
Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

- **CONTENT**
- **SYSTEM**
- **REAGENT**
- **CALIBRATOR**

---

**FOR US CUSTOMERS ONLY: LIMITED WARRANTY**
Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

COBAS, COBAS E, ELECSYS, MODULAR and PRECICONTROL are trademarks of Roche. INTRALIPID is a trademark of Fresenius Kabi AB.

All other product names and trademarks are the property of their respective owners.

Significant additions or changes are indicated by a change bar in the margin.

© 2013, Roche Diagnostics