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Digoxin

cobas®

Digoxin

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
11820796 322	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay 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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay 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 toxic 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[a)].

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (10 µL) with a digoxin-specific ruthenium-labeled antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and a digoxin derivative labeled with biotin, the still-vacant sites of the ruthenium labeled antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- 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 rackpack is labeled as DIGO.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-digoxin-Ab~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 10 mL:
Monoclonal anti-digoxin antibody (mouse) labeled with ruthenium complex 15 µg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 Digoxin-derivative~biotin (black cap), 1 bottle, 10 mL:
Biotinylated digoxigenin 1.06 ng/mL; biotin 15 µg/L; phosphate buffer 100 mmol/L, pH 7.0; 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 foam formation in all reagents and sample types (specimens, calibrators and controls).

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 barcodes.

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.

Li-, Na-, NH_4^+-heparin and K_3-EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

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

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)

- REF 11820907322, Digoxin CalSet, 4 x 1.5 mL
- REF 04917049190, PreciControl Cardiac II, for 2 x 2 mL each of PreciControl Cardiac II 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 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** 411 analyzers:

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

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

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

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- REF 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 < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 409 nmol/L or < 100 ng/mL).

Criterion: 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 rheumatoid 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, nabumetone, 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 80000 ng/mL.

Digoxin-like immunoreactive substances (DLIS) 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 DLIS in a sample can result in a false elevation of digoxin when assayed by commercially available immunoassays.[10,11,12]

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.

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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.[14] Some overlap of toxic and non-toxic values has been reported.[3] 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 (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HS[b] 1	0.68	0.53	0.03	0.02	4.4	0.05	0.04	7.8
HS 2	1.91	1.49	0.05	0.04	2.8	0.08	0.06	4.3
HS 3	4.10	3.20	0.12	0.09	2.9	0.15	0.12	3.7
HS 4	5.90	4.61	0.14	0.11	2.4	0.20	0.15	3.3
PC[c] CARD1	1.48	1.16	0.05	0.04	3.4	0.07	0.06	5.0
PC CARD2	3.64	2.84	0.10	0.08	2.9	0.15	0.11	4.0

b) HS = human serum

c) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HS 1	0.65	0.50	0.04	0.03	5.6	0.06	0.05	9.8
HS 2	1.84	1.44	0.05	0.04	3.0	0.10	0.08	5.3
HS 3	4.05	3.16	0.10	0.08	2.5	0.15	0.12	3.7
HS 4	5.70	4.45	0.14	0.11	2.5	0.18	0.14	3.2
PC CARD1	1.52	1.19	0.05	0.04	3.0	0.09	0.07	5.9
PC CARD2	3.73	2.91	0.09	0.07	2.5	0.14	0.11	3.7

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[15]	Linear regression
y = 1.06x - 0.06	y = 1.09x - 0.08
τ = 0.999	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:

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

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

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

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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 integral 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	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

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