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Digitoxin

cobas®

Digitoxin

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
03002659 122	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of digitoxin in human serum and plasma. The measurement is used for identifying and treating digitoxin intoxication and monitoring digitoxin levels to support a 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

Digitoxin belongs to the family of cardio-active glycoside drugs. Its exact mode of action has not been fully explained. A dose of digitoxin eventually increases the intracellular concentration of Ca^{2+}, which is extremely important for cardiac muscle function.[1] As a result, the heart's ability to contract increases and its contraction amplitude increases, resulting in a greater volume of blood being transported with each beat of the heart.[1]

Digitoxin is used to treat cardiac insufficiency. Additional indications include the treatment of pronounced arterial hypertension in older patients, in preoperative settings for hypertension patients with coronary insufficiency, and for treatment of angina pectoris patients with an enlarged heart and a tendency for distolic ventricular pressure to increase. Digitoxin in contraindicated in patients with severe ventricular arrhythmia and certain forms of pericarditis.

The therapeutic range of digitoxin is approximately 13-39 nmol/L (10-30 ng/mL).[2] In this case, digitoxin exhibits much stronger protein binding than digoxin.[3,4] Since the digitalis effect depends on a number of factors, the therapeutic and the toxic concentration range can overlap.[1] Serum values should therefore be interpreted only within the context of the entire clinical picture.[5]

Digitoxin is eliminated with a half life of 6-8 days. It is metabolized largely in the liver. In this process, digoxin is produced out of about 10 % of the administered dose. Approximately 30 % of the digitoxin administered is eliminated renally.[6]

Determination of the serum concentration is indicated, e.g. for therapy monitoring, for monitoring a patient's dosing regimen, for confirming suspicion of intoxication, in patients with changed pharmacokinetics or unknown premedication, and in patients in which a digitalis effect was not detected in an ECG (electrocardiogram).[2,5,6,7]

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (15 µL) with a digitoxin-specific biotinylated 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 digitoxin labeled with a ruthenium complex[a)], the still-vacant sites of the biotinylated 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.

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

Reagents - working solutions

The reagent rackpack is labeled as DIGIT.

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

R1 Anti-digitoxin-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated polyclonal anti-digitoxin antibody (sheep) 0.5 mg/L; phosphate buffer 100 mmol/L, pH 6.8; preservative.

R2 Digitoxin~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Digitoxin, labeled with ruthenium complex 4.0 µg/L; phosphate buffer 100 mmol/L, pH 6.8; 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	4 weeks

Specimen collection and preparation

Samples for digitoxin analyses should preferably be collected 8-24 hours after administration of the drug.[1]

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, K_3-EDTA and sodium citrate 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 1 week at 2-8 °C, 6 months at -20 °C.[8,9] Freeze only once.

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.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

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 03002667122, Digitoxin CalSet, for 4 x 1 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 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

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.

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 approximately 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 against reference standards by weighing digitoxin into analyte-free human serum matrix.

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 8 weeks 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 0.76 = ng/mL
	ng/mL x 1.31 = nmol/L

Limitations - interference

The assay is unaffected by icterus (bilirubin < 633 µmol/L or < 37 mg/dL), hemolysis (Hb < 0.683 mmol/L or < 1.1 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

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 1500 IU/mL.

In vitro tests were performed on a panel of commonly used pharmaceuticals. While 40 of these showed no interference with the assay, uzara, hydrocortisone and canrenone were identified to cause falsely elevated digitoxin values at concentrations of the recommended daily dose.

Due to a cross-reaction of 1.1 %, ouabain can cause falsely elevated digitoxin values. In isolated cases, glycoside concentrations may be found which do not correlate with values to be expected based on the dosage. Causes may be missing patient compliance, for example, or pre-medication which had not been taken into consideration.

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. DLIS can also interfere with digitoxin assays.

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.

Limits and ranges

Measuring range

2.62-105 nmol/L or 2.00-80.0 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 2.62 nmol/L or < 2.00 ng/mL. Values above the measuring range are reported as > 105 nmol/L or > 80 ng/mL (or up to 210 nmol/L or 160 ng/mL for 2-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 2.62 nmol/L or 2.00 ng/mL

The detection limit represents the lowest measurable analyte level that can be distinguished from zero.

Dilution

Samples with digitoxin 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 > 39 nmol/L or > 30 ng/mL.

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

In the literature, the mean therapeutic serum level for digitoxin is indicated as being 13-39 nmol/L or 10-30 ng/mL.[3]

Since the therapeutic and the toxic serum level can overlap, the monitoring of the glycoside levels as well as the clinical findings must be taken into consideration to clarify a possible digitalis intoxication. See also the "Limitations - interference" section.

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		
Sample	Mean		SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%
Human serum 1	6.79	5.19	0.16	0.12	2.3
Human serum 2	25.9	19.8	0.43	0.33	1.7
Human serum 3	46.7	35.6	0.88	0.67	1.9
Human serum 4	88.6	67.7	1.63	1.25	1.8
PC CARDII1	20.0	15.3	0.33	0.26	1.7
PC CARDII2	44.1	33.7	0.63	0.48	1.4

Elecsys 2010 and **cobas e** 411 analyzers					
			Intermediate precision		
Sample	Mean		SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%
Human serum 1	6.79	5.19	0.36	0.28	5.3
Human serum 2	25.9	19.8	0.94	0.72	3.6
Human serum 3	46.7	35.6	1.39	1.06	3.0
Human serum 4	88.6	67.7	2.53	1.93	2.9
PC CARDII1	20.0	15.3	0.68	0.52	3.4
PC CARDII2	44.1	33.7	1.20	0.92	2.7

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
			Repeatability		
Sample	Mean		SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%
Human serum 1	7.16	5.47	0.23	0.17	3.2
Human serum 2	26.6	20.3	0.53	0.40	2.0
Human serum 3	48.2	36.8	0.86	0.66	1.8
Human serum 4	92.6	70.7	2.07	1.58	2.2
PC CARDII1	20.2	15.4	0.41	0.32	2.1

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
			Repeatability		
Sample	Mean		SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%
PC CARDII2	44.7	34.1	0.77	0.59	1.7

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
			Intermediate precision		
Sample	Mean		SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%
Human serum 1	7.16	5.47	0.26	0.19	3.6
Human serum 2	26.6	20.3	0.65	0.50	2.4
Human serum 3	48.2	36.8	1.14	0.87	2.4
Human serum 4	92.6	70.7	2.96	2.26	3.2
PC CARDII1	20.2	15.4	0.55	0.42	2.8
PC CARDII2	44.7	34.1	1.16	0.88	2.6

Method comparison

A comparison of the Elecsys Digitoxin assay (y) with CEDIA Digitoxin (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 82

Passing/Bablok regression[10]

Slope: 0.95 (95 % confidence range: 0.93-1.11)

Intercept: -0.30 (95 % confidence range: -2.88-0.08)

Coefficient of correlation: 0.912

The sample concentrations were between approximately 6.5 and 55 nmol/L (approximately 5 and 42 ng/mL).

Analytical specificity

For the polyclonal antibody used, the following cross-reactivities were found:

	Concentrations tested ng/mL	Cross-reactivity %
Cortisol	2000	n.d. [b)]
Corticosterone	2000	n.d.
DHEA-S	2000	n.d.
Prednisolone	2000	n.d.
Ouabain	2000	1.1
Prednisone	1000	n.d.
Estradiol	200	n.d.
Progesterone	200	n.d.
Proscillaridin	5000	3.6
k-strophanthin	500	40
Testosterone	200	n.d.
Digitoxigenin-mono-digitoxoside	40	153
Digitoxigenin-bis-digitoxoside	40	146
Digitoxigenin	40	220
Dihydrodigoxin	25	n.d.
Digoxigenin-mono-digitoxoside	25	n.d.
Digoxigenin-bis-digitoxoside	25	n.d.
Digoxin	25	n.d.
Digoxigenin	25	n.d.

b) n.d. = not detectable; cross-reactions below the lower detection limit

References

1 Jortani SA, Valdes R Jr. Digoxin and Its Related Endogenous Factors Critical Reviews. Clin Lab Sci 1997;34(3):225-274.

2 Valdes R, Jortani SA, Gheorghiade M. Standards of laboratory practice: cardiac drug monitoring. Clin Chem 1998;44(5):1096-1109.

3 Doherty JE. Digitalis glycosides. Pharmacokinetics and their clinical implications. Ann Internal Med 1973;79:229-238.

4 Perrier D, Mayerson M, Marcus FI. Clinical pharmacokinetics of digitoxin. Clin Pharmacokin 1977;2:292-311.

5 Smith TW. Pharmacokinetics, Bioavailability and Serum Levels of Cardiac Glycosides. J Am Coll Cardiol 1985;5:43A-50A.

6 Oellerich M. Pharmaka (Drug monitoring). In: Thomas L (ed.). Labor und Diagnose, TH-Books, Frankfurt, 5. edition, 1998:1174. Englisch: Clinical Laboratory. 1st English Edition 1998:1151.

7 Beller GA, Smith TW, Abelmann WH, et al. Digitalis Intoxication: A Prospective Clinical Study with Serum Level Correlations. New Engl J Med 1971;284:989-997.

8 Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests, 2nd editon, 1997:3-180.

9 Guder WG, Narayanan S, Wisser H, et al. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. GIT-Verlag, Darmstadt;1996:8. ISBN 3-928865-22-6:32/33.

10 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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

CE

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