

Digitoxin

cobas®

REF		SYSTEM
03002659 122	100	MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

English

System information

For **cobas e 411** analyzer: test number 260
For MODULAR ANALYTICS E170, **cobas e 601** and **cobas e 602** analyzers: Application Code Number 129

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 electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Digitoxin belongs to the family of steroidal cardio-active glycoside drugs which exert a positive inotropic effect by increasing the intracellular Ca^{2+} concentration in cardiac muscle cells.¹

Several molecular mechanisms of drug actions have been discussed,² with the most widely accepted that digitoxin acts by binding and inhibiting the membrane-bound Na^+/K^+ -ATPase which ultimately increases the intracellular Ca^{2+} concentration.^{1,3} As a result, both the heart's ability to contract and its contraction amplitude increase, resulting in a greater volume of blood being transported with each beat of the heart.⁴

Digitoxin is used to treat cardiac insufficiency. Additional indications include 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 diastolic ventricular pressure to increase. Digitoxin is contraindicated in patients with severe ventricular arrhythmia and certain forms of pericarditis.

The therapeutic range of digitoxin is approximately 10-25 ng/mL (13-33 nmol/L)^{5,6,7,8,9} with 30 ng/mL (39 nmol/L) being the threshold to toxicity.^{2,8,9} In general, digitoxin exhibits much stronger protein binding than digoxin.^{6,10,11} As digitoxin has a long distribution phase blood samples should be taken 5-6 hours after the last drug intake.⁵

Since the digitalis effect depends on a number of factors, the therapeutic and the toxic concentration range can overlap. Serum values should therefore be interpreted only within the context of the entire clinical picture.^{5,11,12}

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 by renal excretion.⁷

Determination of the serum concentration is indicated, e.g. for digitalis therapy monitoring, for monitoring a patient's dosing regimen, for confirming suspicion of intoxication, and in patients in which a digitalis effect was not detected in an electrocardiogram.^{6,7,11,13}

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 or e-barcode.

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

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- $\text{Ru}(\text{bpy})_3^{2+}$ (black cap), 1 bottle, 9 mL: Digitoxin, labeled with ruthenium complex 4.0 $\mu\text{g/L}$; 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.⁴

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li^- , Na^- , NH_4^+ -heparin, $\text{K}_3\text{-EDTA}$ and sodium citrate plasma.

Li -heparin plasma tubes containing separating gel can be used.

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

Stable for 1 week at 2-8 °C, 6 months at -20 °C.^{14,15} 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

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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 1.0 mL
- [REF] 04917049190, PreciControl Cardiac II, for 4 x 2.0 mL
- [REF] 11732277122, Diluent Universal, 2 x 16 mL sample diluent or [REF] 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- [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, AssayCup, 60 x 60 reaction cups
- [REF] 11706799001, AssayTip, 30 x 120 pipette tips
- [REF] 11800507001, Clean-Liner

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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- [REF] 03023150001, WasteLiner, waste bags
- [REF] 03027651001, SysClean Adapter M

Accessories for all analyzers:

- [REF] 11298500316, ISE Cleaning Solution/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 (except for the **cobas e** 602 analyzer).

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).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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.

If necessary, repeat the measurement of the samples concerned.

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:

$$\text{nmol/L} \times 0.76 = \text{ng/mL}$$

$$\text{ng/mL} \times 1.31 = \text{nmol/L}$$

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.

As stated by the manufacturers of digitalis antidotes, the therapeutic antibody fragments against digitalis (e.g. DigiFab, DigiBind) will interfere with digitalis immunoassay measurements.¹⁶ Therefore, the manufacturer of DigiFab recommends to obtain samples for determination of digitoxin concentration prior to antidote administration.¹⁶ As a consequence Elecsys Digitoxin concentrations maybe falsely elevated if measured in the presence of the antidote until the Fab fragments are eliminated from the body.¹⁶

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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 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 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 13-33 nmol/L or 10-25 ng/mL.⁹

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.¹⁷ 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 duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
Sample	Mean		Repeatability		
	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

cobas e 411 analyzer					
Sample	Mean		Intermediate precision		
	SD		CV		
	nmol/L	ng/mL	nmol/L	ng/mL	%
Human serum 1	6.79	5.19	0.36	0.28	5.3

cobas e 411 analyzer					
Sample	Mean		Intermediate precision		
	SD		CV		
	nmol/L	ng/mL	nmol/L	ng/mL	%
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					
Sample	Mean		Repeatability		
	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
PC CARDII2	44.7	34.1	0.77	0.59	1.7

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Mean		Intermediate precision		
	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 regression¹⁸

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.
Oubain	2000	1.1

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	Concentrations tested ng/mL	Cross-reactivity %
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

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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 (for USA: see <https://usdiagnostics.roche.com> for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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