DIG

cobas®

ONLINE TDM Digoxin

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05841267190	05841267500	ONLINE TDM Digoxin (200 tests)	System-ID 01 3783 6	**cobas c** 701/702

Materials required (but not provided):

03375790190	Preciset TDM I CAL A-F (6 x 5 mL) Diluent (1 x 10 mL)	Codes 691-696	
04521536190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 310 Code 311 Code 312	

English

System information

DIG: ACN 8081

Intended use

In vitro test for the quantitative determination of digoxin in serum and plasma on **cobas c** systems.

Summary

Digoxin measurements performed with this assay in human serum and plasma are used as an aid to ensure proper therapeutic drug concentrations and to minimize toxic effects.

Digoxin is one of a group of cardiac glycosides obtained from digitalis plants. Cardiac glycosides have long and intensively been used in clinics to treat patients with heart complaints. The most widely accepted mechanism of action of cardiac glycosides (also referred to as digitalis) is the inhibition of the activity of the Na^+K^+-ATPase enzyme. Inhibition of this "sodium pump" is postulated to promote the movement of calcium ions in the cell, thus improving cardiac contractility.[1]

Digoxin can be prescribed for the management of supraventricular arrhythmias, such as atrial fibrillation, and in the management of chronic heart failure, although its use has decreased due to the narrow therapeutic index.[2,3,4,5,6] For this same reason, therapeutic drug monitoring (TDM) of digoxin (and related glycosides, e.g. metabolites) is required to ascertain adherence to digoxin treatment and to verify intoxication.[7] The therapeutic and toxic effects of cardiac glycosides are concentration-dependent.[8,9] Therapeutic dosage needs to be adjusted for each individual and balanced against the disappearance of clinical symptoms of the underlying disease and the non-induction of major side effects.[10] In patients with comorbidities (i.e. chronic kidney disease) or other factors affecting digoxin metabolism (including other drugs) and/or the elderly, measurements of serum digoxin concentrations are recommended.[5,6] Dosage reduction is required in patients with renal impairment since digoxin is primarily excreted by glomerular filtration. Measurement of digoxin can be useful as a predictor for digoxin toxicity, but concentrations need to be interpreted in the overall clinical context.[9] In the case of acute intoxication, measurement is also useful to determine antidote dosage.[11]

Test principle

Kinetic interaction of microparticles in solution (KIMS) as measured by changes in light transmission.

The Digoxin assay is a homogeneous immunoassay based on the principle of measuring changes in scattered light or absorbance which result when activated microparticles aggregate. The microparticles are coated with digoxin and rapidly aggregate in the presence of a digoxin antibody solution. When a sample containing digoxin is introduced, the aggregation reaction is partially inhibited, slowing the rate of the aggregation process. Antibody bound to sample drug is no longer available to promote microparticle aggregation, and subsequent particle lattice formation is inhibited. Thus, a classic inhibition curve with respect to digoxin concentration is obtained, with the maximum rate of aggregation at the lowest digoxin concentration. By monitoring the change in scattered light or absorbance, a concentration-dependent curve is obtained.

Reagents - working solutions

R1 Anti-digoxin monoclonal antibody (mouse) and human-sourced material in buffer with preservative

R3 Conjugated digoxin derivative microparticles, human-sourced material, and preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A). However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[12,13]

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	12 weeks
On-board on the Reagent Manager:	24 hours

Do not freeze.

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

A specimen should be collected at least 6 to 8 hours after drug administration.[14] By this time, serum digoxin levels are expected to be in equilibrium with tissue levels and should correlate with pharmacologic effects.

Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sampling tubes.
Plasma: Li-heparin plasma and K_2-EDTA plasma.

Stability:[15]	24 hours capped at 2-8 °C 1-2 weeks capped at -20 °C (± 5 °C)

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.

See the limitations and interferences section for details about possible sample interferences.

Invert thawed specimens several times prior to testing.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

cobas c 701/702 **test definition**

Assay type	2-Point End		
Reaction time /Assay points	10 / 22-38		
Wavelength (sub/main)	– /660 nm		
Reaction direction	Increase		
Unit	ng/mL (nmol/L)		
Reagent pipetting		Diluent (H_2O)	
R1	84 µL	–	
R3	22 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	5.5 µL	–	–
Decreased	5.5 µL	–	–
Increased	5.5 µL	–	–

Calibration

Calibrators	S1-6: Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	6-point calibration - after lot change - as required following quality control procedures

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

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of digoxin in normal human serum.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

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

cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factor:[16] ng/mL x 1.28 = nmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a digoxin level of approximately 2.5 ng/mL (3.2 nmol/L).

Icterus:[17] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).

Hemolysis:[17] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid):[17] No significant interference up to an L index of 850. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 100 IU/mL.

Total protein: No significant interference from total protein up to a concentration of 14 g/dL.

There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.

In rare instances (< 1 %), samples contain unidentified components which cause nonspecific agglutination in this assay. These samples give falsely lowered digoxin values. If a result is obtained which is inconsistent with the patient's clinical picture, contact Customer Technical Support.

As with all digoxin immunoassays, Digibind therapy for digoxin toxicity will interfere with digoxin measurement by this assay.

As with many mouse monoclonal antibody-based immunoassays, this assay may experience interference with samples containing human anti-mouse antibodies (HAMA). Samples suspected of containing HAMA (e.g., from patients with history of mouse monoclonal antibody exposure) should be tested by an alternate method.

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

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.3-5.0 ng/mL (0.38-6.4 nmol/L) (defined by the Limit of Detection and the upper limit of linearity).

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM I Diluent (0 ng/mL) and reassay. Multiply the result by 2 to obtain the specimen value.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation/functional sensitivity

Limit of Blank = 0.2 ng/mL (0.26 nmol/L)

Limit of Detection = 0.3 ng/mL (0.38 nmol/L)

Limit of Quantitation = 0.4 ng/mL (0.51 nmol/L)

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Quantitation was determined using the result of functional sensitivity testing.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest Digoxin concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20 %.

Values below the Limit of Detection (< 0.3 ng/mL) will not be flagged by the instrument.

Expected values

Accurate determination of a patient's sample digoxin concentration is necessary because of the extremely narrow therapeutic range of this drug. In addition, the significant variability of patient response even under similar dosing regimens often produces unpredictable responses in serum digoxin concentrations.[18] Ratios of heart/serum digoxin levels may vary between 17:1 and 35:1.[19]

A relationship between serum levels of digoxin and therapeutic or toxic effects has been demonstrated in numerous studies.[20,21,22] Therapeutic effects are seen with concentrations between approximately 0.8 and 2 ng/mL (1.0 and 2.6 nmol/L). Serum digoxin concentrations above 2 ng/mL (2.6 nmol/L) are associated with symptoms of toxicity, while concentrations less than 0.8 ng/mL (1.0 nmol/L) are generally not effective.[23]

Based on actual new *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 (0.77-1.5 nmol/L) is recommended.[24] Increased risk of mortality was observed for digoxin concentration of 1.2 ng/mL (1.5 nmol/L) and higher.[25]

The evaluation of test results should consider additional factors including age, renal function, and clinical symptoms of the patient.[20,21,22]

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and/or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

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[17]

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

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (n = 63). The following results were obtained on the **cobas c** 701 analyzer:

Repeatability	*Mean*		*SD*		*CV*
	ng/mL	*nmol/L*	*ng/mL*	*nmol/L*	*%*
TDM Control Level 1	1.03	1.32	0.02	0.03	2.0
TDM Control Level 2	1.93	2.47	0.02	0.03	0.9
TDM Control Level 3	3.32	4.25	0.03	0.04	0.8
Human serum A	0.811	1.04	0.014	0.02	1.7
Human serum B	1.46	1.87	0.02	0.03	1.4
Human serum C	3.95	5.06	0.03	0.04	0.7

Intermediate precision	*Mean*		*SD*		*CV*
	ng/mL	*nmol/L*	*ng/mL*	*nmol/L*	*%*
TDM Control Level 1	0.873	1.12	0.052	0.07	6.0
TDM Control Level 2	1.77	2.27	0.04	0.05	2.4
TDM Control Level 3	3.03	3.88	0.05	0.06	1.6
Human serum 1	1.15	1.47	0.04	0.05	3.2
Human serum 2	2.17	2.78	0.05	0.06	2.2

Results for intermediate precision were obtained on the **cobas c** 501 analyzer.

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 701 analyzer(s).

Method comparison

Digoxin values for human serum and plasma samples obtained on a **cobas c** 701 analyzer (y) were compared with those determined using the corresponding reagent on the **cobas c** 501 analyzer (x).

Sample size (n) = 82

Passing/Bablok[26]	Linear regression
y = 0.964x + 0.060 ng/mL	y = 0.963x + 0.063 ng/mL
τ = 0.924	r = 0.996

The sample concentrations were between 0.32 and 4.82 ng/mL (0.41 and 5.17 nmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration Tested (ng/mL)**	**% Cross-reactivity**
β-Acetyldigoxin	2.0	82.5
Digitoxin	48.8	4.5
Digitoxigenin	39	1.2
Digoxigenin	25	8.6
Digoxigenin bis-digitoxose	2	130
Digoxigenin mono-digitoxose	2	107.5
Dihydrodigoxin	20	6.5
β-Methyldigoxin	1	115
Dehydroisoandrosterone	10000	ND
Digitoxose	10000	ND
Estradiol	10000	ND
Estriol	10000	ND
Hydrocortisone	10000	ND
11-Hydroxyprogesterone	10000	ND
17-Hydroxyprogesterone	10000	ND
Prednisolone	10000	ND
Prednisone	10000	ND
Progesterone	10000	ND
Spironolactone	75000	ND

ND = Not detectable

Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa + 1.5 H_2O
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin

Cyclosporine Theophylline

References

1 Milone MC, Shaw LM. Therapeutic Drugs and Their Management, In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 42, p. 420-453.e9.

2 Aspen Pharma Trading Limited, Digoxin 0.0625mg tablets – Drug information [revised 2022 June; cited 2023 August 25]. Available from: https://www.medicines.org.uk/emc/product/5464/smpc.

3 January CT, Wann LS, Calkins H, et al. 2019 AHA/ACC/HRS Focused Update of the 2014 AHA/ACC/HRS Guideline for the Management of Patients With Atrial Fibrillation: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society in Collaboration With the Society of Thoracic Surgeons. Circulation 2019 Jul 9;140(2):e125-e151.

4 Heidenreich PA, Bozkurt B, Aguilar D, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation 2022 May 3;145(18):e895-e1032.

5 McDonagh TA, Metra M, Adamo M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2921 Sep 21;42(36):3599-3726.

6 Macle L, Cairns J, Leblanc K, et al. 2016 Focused Update of the Canadian Cardiovascular Society Guidelines for the Management of Atrial Fibrillation. Can J Cardiol 2016 Oct;32(10):1170-1185.

7 Orrico KB, Wu M, Wilson AR. Assessment of the appropriateness of serum digoxin concentration measurement in a medical group setting. J Manag Care Pharm 2011 Nov;17(9):695-700.

8 Kanji S, MacLean RD. Cardiac glycoside toxicity: more than 200 years and counting. Crit Care Clin 2012 Oct;28(4):527-535.

9 Jürgens G, Graudal NA, Kampmann JP. Therapeutic drug monitoring of antiarrhythmic drugs. Clin Pharmacokinet 2003;42(7):647-663.

10 Smith TW. Pharmacokinetics, bioavailability and serum levels of cardiac glycosides. J Am Coll Cardiol 1985 May;5(5 Suppl A):43A-50A.

11 Hassan SA, Goyal A. Digoxin Immune Fab 2022 Nov 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–.

12 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

13 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

14 Jortani SA, Valdes R Jr. Digoxin and its related factors. Crit Rev Clin Lab Sci 1997;34(3):225-274.

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

16 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;46.

17 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

18 Lindenbaum J, Mellow MH, Blackstone MO, et al. Variation in the biologic availability of digoxin from four preparations. New Engl J Med 1971;285:1344-1347.

19 Doherty JE, Perkins WH, Flanigan WJ. The distribution and concentration of titrated digoxin in human tissues. Ann Intern Med 1967;66:116.

20 Smith TW, Haber E. Digoxin intoxication: the relationship of clinical presentation to serum digoxin concentration. J Clin Invest 1970;49:2377-2386.

21 Reuning RH, Sams RA, Notari RE. Role of pharmacokinetics in drug dosage adjustment. I. Pharmacokinetic effect and apparent volume of distribution of digoxin. J Clin Pharmacol 1973;13:127-141.

22 Whiting B, Sumner DJ, Goldberg A. An assessment of digoxin radioimmunoassay. Scott Med J 1973;18:69-74.

23 Huffman DH, Crow JW, Pentikaimen P, et al. Clinical cardiac status, laboratory parameters and digoxin usage. Am Heart J 1976;91:28.

24 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.

25 Rathore SS, Curtis JP, Wang Y, et al. Association of serum digoxin concentration and outcomes in patients with heart failure. JAMA 2003;289(7):871-878.

26 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.

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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

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

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606