

Digoxin

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
20737836 322	Digoxin (250 tests)	System-ID 07 3783 6 COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375790 190	Preciset TDM I Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6830 8
04521536 190	TDM Control Set Level I (2 × 5 mL) Level II (2 × 5 mL) Level III (2 × 5 mL)	System-ID 07 6900 2 System-ID 07 6901 0 System-ID 07 6902 9

English

System information

Test DIGM, test ID 0-283

Intended use

In vitro diagnostic test for the quantitative determination of digoxin in serum or heparinized plasma on COBAS INTEGRA systems.

Summary

Digoxin is a digitalis glycoside that exerts a positive inotropic effect that subsequently increases the contractile response of the myocardial fibers in patients experiencing congestive heart failure.¹ Cardiac glycosides also can produce several electrophysiologic effects that produce negative chronotropic effects on the human heart.² These effects tend to slow down and regulate a rapid, irregular beat like that found in patients experiencing cardiac arrhythmias.³

Test principle

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

The COBAS INTEGRA Digoxin test 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** Antibody reagent
Anti-digoxin monoclonal antibody (mouse) and human-sourced material in buffer with preservative.
- SR** Microparticle reagent
Conjugated digoxin derivative microparticles, human-sourced material, and preservative.

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

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

COBAS INTEGRA 400 plus analyzers

The **cobas c** pack has to be mixed daily before use. Place the **cobas c** pack on the Cassette Mixer and mix for 1 minute.

COBAS INTEGRA 800 analyzers

The reagent is automatically mixed for 2 minutes after **cobas c** pack puncture and for half a minute during Begin of Day.

Storage and stability

Shelf life at 2-8 °C

See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 10 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 26 weeks

The on-board in use stability period begins at the time of **cobas c** pack puncture.

Specimen collection and preparation

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

Only the specimens listed below were tested and found acceptable:

Unhemolyzed serum

Unhemolyzed heparinized plasma

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.

A specimen should be collected 6-8 hours following the last oral dose of digoxin. By this time, serum digoxin levels are expected to be in equilibrium with tissue levels and should correlate with pharmacologic effects.

Prior to analysis, the serum may be stored refrigerated (2-8 °C) for up to 24 hours or at -20 °C for 1-2 weeks.⁴ Specimens should not be repeatedly frozen and thawed. Any additional clotting or precipitation which occurs due to the freeze/thaw treatment should be removed by centrifugation prior to analyzing the digoxin concentration of that sample.

Materials provided

See "Reagents – working solutions" section for reagents.

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.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Reaction mode	R1-S-SR
Wavelength A	652 nm
Reading cycle blank/test	34/65
Unit	ng/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	84 µL	6 µL
Sample	5.5 µL	6 µL
SR	22 µL	6 µL
Total volume	129.5 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Reaction mode	R1-S-SR
Wavelength A	659 nm
Reading cycle blank/test	45/98
Unit	ng/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	84 µL	6 µL
Sample	5.5 µL	6 µL
SR	22 µL	6 µL
Total volume	129.5 µL	

Calibration

Calibrators	Preciset TDM I Calibrators A-F
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at 1 ng/mL (1.3 nmol/L)
Calibration interval	
COBAS INTEGRA 400 plus analyzers	Each lot, every 14 days, and as required following quality control procedures
COBAS INTEGRA 800 analyzers	Each lot, every 12 weeks, and as required following quality control procedures

A calibration curve must be prepared using the Preciset TDM I calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The Preciset TDM I calibrators are prepared to contain known quantities of digoxin in normal human serum and are traceable to USP reference standards.

Note

Calibrators should be assayed within 2 hours after placing on-board the instrument.

Quality control

Quality control	TDM Control Set
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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.

Note

Controls should be assayed within 2 hours after placing on-board the instrument.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: ng/mL × 1.28 = nmol/L

Limitations - interference

See the Analytical specificity section of this method sheet for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

Specimens with assay values greater than the highest calibrator are flagged by the system. To obtain a specimen digoxin concentration value if this occurs, dilute the original specimen manually with the Preciset TDM I diluent (0 ng/mL), reassay, and multiply the result by the appropriate dilution factor.

Certain samples have been observed to cause nonspecific microparticle aggregation in the COBAS INTEGRA Digoxin assay. As a precaution, samples flagged as < 0.2 ng/mL (< 0.26 nmol/L) TEST RANGE on all COBAS INTEGRA analyzers should be re-assayed and the absorbance unit change (ΔA) obtained. Refer to the COBAS INTEGRA systems User Manual, Operation-Results for instructions regarding how to access raw data. The observed ΔA should be compared to the ΔA of the 0 ng/mL calibrator, obtained by highlighting (as described above) the calibrator in the curve results. The Results window must be in the "View - By Time Complete" mode in order to view the calibration data. Samples with absorbance unit changes (ΔA) of more than 0.020 above the 0 ng/mL calibrator rate should be retested by another established method before reporting digoxin results. In rare instances (< 1 %), the nonspecific aggregation could cause erroneously low but unflagged results. Any digoxin result which is inconsistent with the clinical presentation should be confirmed by retesting with an alternate method.

Uzara and Pentoxifylline were identified to cause falsely elevated digoxin values at concentrations of the recommended daily dose.

Hydrocortisone does not interfere at concentrations of the recommended daily dose, however, at higher doses, as administered in life-threatening situations, hydrocortisone may cause elevated digoxin values.

Endogenous substances such as DLIF (digoxin-like immunoreactive factors) may interfere with this assay by yielding lightly elevated results.^{5,6,7} DLIF are observed primarily in samples from neonates, pregnant women, and acute care patients with renal or hepatic failure.

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.⁸

Falsely elevated digoxin values might be obtained in patients undergoing digitoxin therapy.

As with many mouse monoclonal antibody-based immunoassays, the COBAS INTEGRA Digoxin **cobas c** pack 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.

Serum/plasma

Criterion: Recovery within ± 10 % of initial value at a digoxin concentration of 1 ng/mL (1.28 nmol/L).

Icterus:⁹ No significant interference up to a bilirubin concentration of 431 µmol/L or 25.2 mg/dL.

Hemolysis:⁹ No significant interference up to a hemoglobin concentration of 2000 mg/dL.

Lipemia:⁹ No significant interference up to a triglycerides concentration of 2400 mg/dL.

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

Specimens containing bilirubin, triglycerides, and/or hemoglobin at levels above those listed above should be diluted with the Preciset TDM I diluent (0 ng/mL), assayed, and the results multiplied by the appropriate dilution factor.

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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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).

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation/functional sensitivity (LoQ)

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 $n \geq 60$ 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 analyte concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20 %. It has been determined using low concentration digoxin samples.

Note: For COBAS INTEGRA analyzers, values between 0.2-0.3 ng/mL (0.26-0.38 nmol/L) 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.¹⁰ Ratios of heart/serum digoxin levels may vary between 17:1 and 35:1.¹¹

A relationship between serum levels of digoxin and therapeutic or toxic effects has been demonstrated in numerous studies.^{12,13,14} Therapeutic effects are seen with concentrations between approximately 0.8-2 ng/mL (1.0-2.6 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.¹⁵

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.¹⁶ Increased risk of mortality was observed for digoxin concentration of 1.2 ng/mL (1.5 nmol/L) and higher.¹⁷

The evaluation of test results should consider additional factors including age, renal function, and clinical symptoms of the patient.^{12,13,14}

Studies performed on the COBAS FARA II analyzer, using cassette COBAS INTEGRA Digoxin reagents have shown the immunoreactivity of digoxin and its primary metabolites (digoxigenin-bis-digitoxoside, digoxigenin mono-digitoxoside, and digoxigenin) parallel the cardioactivity of these compounds.¹⁸

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 COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using controls in accordance with the NCCLS EP5-T2¹⁹ requirements with repeatability ($n = 80$) and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained on a COBAS INTEGRA 700 analyzer:

Repeatability	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	0.9 (1.1)	0.06 (0.08)	6.8
Level 2	1.6 (2.1)	0.08 (0.10)	5.0
Level 3	2.8 (3.6)	0.10 (0.13)	3.4

Intermediate precision	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	0.9 (1.1)	0.08 (0.10)	9.7
Level 2	1.6 (2.1)	0.10 (0.13)	6.1
Level 3	2.8 (3.6)	0.11 (0.14)	3.9

Method comparison

Digoxin values for human serum samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Digoxin reagent (y) were compared with those determined using commercially available methods (x).

		AxSYM Digoxin II	TDx
Sample size (n)		88	63
Range of values	min.	0.37 ng/mL	0.08 ng/mL
	max.	2.94 ng/mL	2.90 ng/mL
Slope		0.858	0.981
Intercept		0.142 ng/mL	0.281 ng/mL
Correlation coefficient		0.959	0.967

Analytical specificity

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with digoxin at 1.9 ng/mL (2.4 nmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.²⁰ The imprecision of the assay was taken into account when determining cross-reactivity.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result} - \text{analyte concentration})}{\text{concentration of interferent}}$$

Drug	Level tested ng/mL	Cross-reactivity %
β-Acetyldigoxin	2.0	84.0
Digitoxin	48.8	7.7
Digitoxigenin	39	1.2
Digoxigenin	25	6.2
Digoxigenin bis-digitoxose	2	115.0
Digoxigenin mono-digitoxose	2	112.2
Dihydrodigoxin	20	3.0
β-Methyldigoxin	1	110.3

Previously, the following structurally related or potentially co-administered compounds were tested on the COBAS FARA II analyzer using normal human serum spiked with digoxin at 2 ng/mL (2.6 nmol/L).

Drug	Level tested ng/mL	Cross-reactivity %
Canrenone	10000	< 0.001
Dehydroisoandrosterone	10000	< 0.03
Digitoxose	10000	< 0.03
Estradiol	10000	< 0.03
Estriol	10000	< 0.03
Hydrocortisone	10000	< 0.03

Drug	Level tested ng/mL	Cross-reactivity %
11-Hydroxyprogesterone	10000	< 0.03
17-Hydroxyprogesterone	10000	< 0.03
Prednisolone	10000	< 0.03
Prednisone	10000	< 0.03
Progesterone	10000	< 0.03
Spironolactone	10000	< 0.03

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

References

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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



Volume after reconstitution or mixing

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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.

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