

## Digitoxin

### Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
20753599 322	Digitoxin (200 tests)	System-ID 07 5359 9 COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375781 190	Preciset TDM II Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6829 4
20766658 322	Roche-TDM OnLine Digitoxin Controls Level I (2 × 5 mL) Level II (2 × 5 mL) Level III (2 × 5 mL)	System-ID 07 6665 8 System-ID 07 6666 6 System-ID 07 6667 4

### English

#### System information

Test DIGTM, test ID 0-559

#### Intended use

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

#### Summary

Digitoxin 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. Cardiac glycosides also can produce several electrophysiologic effects that produce negative chronotropic effects on the human heart. These effects tend to slow down and regulate a rapid, irregular beat like that found in patients experiencing cardiac arrhythmias.<sup>1</sup>

#### Test principle

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

The COBAS INTEGRA Digitoxin 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 digitoxin and rapidly aggregate in the presence of a digitoxin antibody solution. When a sample containing digitoxin 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 digitoxin concentration is obtained, with the maximum rate of aggregation at the lowest digitoxin concentration. By monitoring the change in scattered light or absorbance, a concentration-dependent curve is obtained.

#### Reagents - working solutions

- R1** Antibody reagent  
Anti-digitoxin monoclonal antibody (mouse) in buffer with preservative.
- SR** Microparticle reagent  
Conjugated digitoxin 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.

For USA: For prescription use only.

#### 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 8 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 26 weeks

#### 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 6-12 hours following the last oral dose of digitoxin.<sup>2</sup> By this time, serum digitoxin 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 24 hours or at -20 °C for 1-2 weeks.<sup>3</sup> Specimens should not be repeatedly frozen and thawed.

Invert thawed specimens several times prior to testing.

Any additional clotting or precipitation which occurs due to the freeze/thaw treatment should be removed by centrifugation prior to analyzing the digitoxin 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	659 nm
Reading cycle blank/test	34/65
Unit	ng/mL

## Pipetting parameters

		Diluent (H <sub>2</sub> O)
R1	105 µL	6 µL
Sample	4.5 µL	6 µL
SR	38 µL	6 µL
Total volume	165.5 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 <sub>2</sub> O)
R1	105 µL	6 µL
Sample	4.5 µL	6 µL
SR	38 µL	6 µL
Total volume	165.5 µL	

## Calibration

Calibrators	Preciset TDM II Calibrators A-F
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 7.5 ng/mL (9.8 nmol/L)
Calibration interval	
COBAS INTEGRA 400 plus analyzers	Each <b>cobas c</b> pack, every 1 week 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 II 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 II calibrators are prepared to contain known quantities of digitoxin 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	Roche-TDM OnLine Digitoxin Controls
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 × 1.31 = 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 (65 ng/mL, 85 nmol/L) are flagged by the system. To obtain a specimen digitoxin concentration value if this occurs, dilute the original specimen manually with the Preciset TDM II 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 Digitoxin assay. As a precaution, samples flagged as < 3.0 ng/mL (< 3.9 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 of the sample should be compared to the ΔA of the 0 ng/mL calibrator. Samples with absorbance unit changes (ΔA) of ≥ 0.023 above the 0 ng/mL calibrator rate should be retested by another established method before reporting digitoxin results.

Uzara, potassium canrenoate and spironolactone were identified to cause falsely elevated digitoxin 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 digitoxin values.

The manufacturer of Digoxin Immune Fab (Antibody fragment therapy) has stated that no immunoassay technique is suitable for quantitating digitoxin in serum from patients undergoing this treatment.<sup>4</sup>

As with many mouse monoclonal antibody-based immunoassays, the COBAS INTEGRA Digitoxin test 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 digitoxin concentration of 12.3 ng/mL (16.1 nmol/L).

Icterus:<sup>5</sup> No significant interference up to a bilirubin concentration of 325 µmol/L or 19 mg/dL.

Hemolysis:<sup>5</sup> No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Lipemia:<sup>5</sup> No significant interference up to a triglycerides concentration of 1112 mg/dL.

Total protein: No significant interference up to a total protein concentration of 12 g/dL.

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

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>6</sup>

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

5-65 ng/mL (6.6-85.2 nmol/L) (defined by the limit of detection and the upper limit of linearity).

### Lower limits of measurement

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

Limit of Blank = 3 ng/mL (3.9 nmol/L)

Limit of Detection = 5 ng/mL (6.6 nmol/L)

Limit of Quantitation = 6 ng/mL (7.9 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 95<sup>th</sup> 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  $\leq 20$  %. It has been determined using low concentration digitoxin samples.

## Expected values

Accurate determination of a patient's sample digitoxin concentration is encouraged due to the narrow therapeutic range of this drug. In addition, the significant variability of patient response even under similar dosing regimens often produces unpredictable levels of serum digitoxin concentrations.

Extensive data are available which demonstrate a relationship between serum digitoxin levels and therapeutic effectiveness.<sup>7</sup> Therapeutic effects are seen with concentrations between approximately 10-30 ng/mL (13-39 nmol/L). Serum digitoxin concentrations above 35 ng/mL (46 nmol/L) are associated with symptoms of toxicity, while concentrations below 10 ng/mL (13 nmol/L) are generally not effective.<sup>7,8,9</sup> However, analysis of serum concentrations alone is not efficient for optimization of digitoxin therapy. Additional factors such as age, thyroid condition, electrolyte balance, hepatic and renal functions, and other clinical symptoms must be considered.<sup>10</sup>

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-T2<sup>11</sup> 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 400 analyzer:

Repeatability	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	15.2 (19.8)	1.41 (1.85)	9.3
Level 2	33.3 (43.3)	0.93 (1.22)	2.8
Level 3	47.4 (62.1)	0.88 (1.15)	1.9

Intermediate precision	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	15.2 (19.8)	1.71 (2.24)	11.3
Level 2	33.3 (43.3)	1.52 (1.99)	4.6
Level 3	47.4 (62.1)	1.39 (1.82)	2.9

## Method comparison

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

	FPIA
Number of samples	231
Range of values	min. 0.57 ng/mL max. 45.3 ng/mL
Slope	0.935
Intercept	1.36 ng/mL
Correlation coefficient	0.968

## Analytical specificity

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with digitoxin at 25.8 ng/mL (33.8 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.<sup>12</sup> The imprecision of the assay was taken into account when determining cross-reactivity. Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.

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

Drug	Level tested ng/mL	Cross-reactivity %
Digoxin	25	ND
Digoxigenin	5	ND
Digoxigenin-mono-digitoxoside	25	13.4
Digoxigenin-bis-digitoxoside	5	ND
Dihydrodigoxigenin	25	ND
Digitoxigenin	25	309
Digitoxigenin-mono-digitoxoside	25	156
Digitoxigenin-bis-digitoxoside	25	129
Dihydrodigitoxigenin	25	12.2
Ouabain	1000	0.4
Testosterone	25	ND
Prednisone	25	ND

ND = Not Detectable

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

## References




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- 10 Physicians' Desk Reference 48th Edition Montvale, NJ: Medical Economics Data Production Inc 1994;1215-1216.
- 11 National Committee for Clinical Laboratory Standards. User Evaluation of Precision Performance of Clinical Chemistry Devices; Tentative Guideline. Villanova, PA.: NCCLS;1992;4(12). NCCLS Publication EP5-T2.
- 12 National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. Villanova, PA.: NCCLS; 1986;6(13). NCCLS Publication EP7-P.

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:

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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