# Application Sheet

Laboratory Name
**Test Name: Digoxin**

**System information**

For **cobas c** 311/501 analyzers:
**DIG:** ACN 81

**Intended use**

In vitro test for the quantitative determination of digoxin in serum and plasma on Roche/Hitachi **cobas c** 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 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

**R2** Conjugated digoxin derivative microparticles, human-sourced material, and preservative

R1 is in position B and R2 is in position C.

**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.
For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.
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 applied were FDA-approved or cleared in compliance with the European Directive 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.[4,5]

**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: | 26 weeks |

**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.[6] 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:[7] 24 hours capped at 2-8 °C
1-2 weeks capped at -20 °C

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.
Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.
Specimens should not be repeatedly frozen and thawed.
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
In addition, other suitable control material can be used.

**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** 501/502 **test definition**

| | | | |
|---|---|---|---|
| Assay type | 2-Point End | | |
| Reaction time /Assay points | 10 / 13-47 | | |
| Wavelength (sub/main) | – /660 nm | | |
| Reaction direction | Increase | | |
| Unit | ng/mL | | |
| Reagent pipetting | | Diluent ($H_2O$) | |
| R1 | 84 µL | – | |
| R2 | 22 µL | 20 µL | |
| *Sample volumes* | *Sample* | *Sample dilution* | |
| | | *Sample* | *Diluent (NaCl)* |
| 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<br>• after lot change<br>• 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**

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy

Refer to Brown Clinic Quality Control Specialty and Subspecialty Policy

**Calculation**

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor:[8] 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).
*Serum/Plasma*
Icterus:[9] 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:[9] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).
Lipemia (Intralipid):[9] 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. 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 slightly elevated results.[10,11,12] 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.[13] Falsely elevated digoxin values might be obtained in patients undergoing digitoxin therapy.
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.
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[14]
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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases.

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

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**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.

**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 95$^{th}$ 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 %. It has been determined using low concentration digoxin samples.

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**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.[15]
Ratios of heart/serum digoxin levels may vary between 17:1 and 35:1.[16]
A relationship between serum levels of digoxin and therapeutic or toxic effects has been demonstrated in numerous studies.[17,18,19] 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.[20]
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.[21]
Increased risk of mortality was observed for digoxin concentration of 1.2 ng/mL (1.5 nmol/L) and higher.[22]
The evaluation of test results should consider additional factors including age, renal function, and clinical symptoms of the patient.[17,18,19]
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**

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

**References**

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**Alternative method**

Refer to Brown Clinic Back-up Testing Policy

**Source document**

Reagent Name: DIG

**Method Sheet Version: V13.0 English Order information**

| REF | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|
| **20737836** 322 | ONLINE TDM Digoxin (250 tests) | System-ID07 3783 6 | Roche/Hitachi **cobas c** 311, **cobas c** 501/502 |
| **03375790** 190 | Preciset TDM I Calibrators<br>CAL A-F (6 x 5 mL)<br>Diluent (1 x 10 mL) | Codes 691-696 | |

## Effective date

Effective date for this procedure:

## Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)$^{cm}$ Date: 4/9/2018

Approved by: Aaron Shives MD (Signature on file Date: 4/11/2018

## REVIEW – REVISION SUMMARY DOCUMENTATION

| Date: | By: | Revision Summary: |
|---|---|---|