Brown Clinic Northridge
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Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Digoxin

PURPOSE:

The DGNA Flex® reagent cartridge used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended to measure digoxin, a cardiovascular drug, in serum and plasma. DGNA test results are used in the diagnosis and treatment of digoxin overdose and in monitoring levels of digoxin to ensure appropriate therapy.

PRINCIPLE:

Digoxin, a cardiac glycoside, is used as an antiarrhythmic agent, both alone and in conjunction with other drugs. Absorption from the gastrointestinal tract is variable: 60-80% of the administered dose is absorbed. Digoxin is excreted by the kidney almost entirely unchanged. Therefore the patient's renal function is an important consideration in determining dosage. In persons with normal kidney function the half-life is about 1.5 days. The most serious complications of digoxin toxicity are ventricular arrhythmias: ventricular tachycardia and ventricular fibrillation.

The digoxin method uses an immunoassay technique in which free and digoxin-bound antibody-enzyme species are separated using magnetic particles. The DGNA chemistry is optimized for measurement of β-galactosidase activity. Magnesium acetate is included to activate the enzyme and N-2-Hydroxyethylpiperazine-N'-1-ethanesulfonic acid (HEPES) buffer to provide optimum pH.

The methodology for DGNA involves Antibody Conjugate reagent mixing with patient's serum or plasma. The Antibody Conjugate reagent utilizes the $F(ab')_2$ fragment of the antibody to eliminate interference from rheumatoid factor. Digoxin in the sample is bound by the $F(ab')_2$-β-galactosidase in the Antibody Conjugate reagent. Magnetic particles coated with the digoxin analog ouabain are added to bind free (unbound) antibody-enzyme conjugate. The reaction mixture is then separated magnetically. Following separation, the supernatant containing the digoxin-antibody-enzyme complex is transferred and mixed with a substrate. The β-galactosidase (β-gal) portion of the Digoxin-F(ab')2-β-galactosidase complex catalyzes the hydrolysis of chlorophenol-β-D-galactopyranoside (CPRG) to chlorophenol red (CPR). The change in absorbance at 577 nm due to the formation of CPR is directly proportional to β-galactosidase activity. Since β-galactosidase is not present in serum, its activity is directly proportional to digoxin in the patient's sample and is measured using a bichromatic (577, 700 nm) rate technique.

Digoxin + F(ab')$_2$-β-galactosidase——> F(ab')$_2$-β-gal + Digoxin-F(ab')$_2$-β-gal

$$\text{Digoxin-F(ab')}_2\text{-β-gal} + \text{F(ab')}_2\text{-β-gal} \xrightarrow[\text{separation}]{\text{magnetic particle}} \text{Digoxin - F(ab')}_2\text{-β-gal}$$

$$\text{CPRG} \xrightarrow{\text{Digoxin-F(ab')}_2\text{-β-gal}} \text{CPR}$$

(non-absorbing at 577 nm) (absorbs at 577 nm)

INSTRUMENT, REAGENTS & SUPPLIES:

Reagents

Wells[a]	Form	Ingredient	Concentration[b]	Source
1,2	Liquid	Antibody Conjugate		rabbit
		Reagent and stabilizers	c	
3,4	Tablets	Ouabain		
		Magnetic Particles	0.3%	
5,6	Tablets	CPRG	7 mM	
7	Liquid	Substrate Diluent,		
		Buffer	100 mM	

a. Wells are numbered consecutively from the wide end of the cartridge.

b. Nominal value per test at manufacture.

c. Antibody titre and conjugate activity vary from lot to lot.

Risk and Safety:

H319, H317

P280, P305 + P351 +P338, P501

Warning!

Causes serious eye irritation. May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautionaly with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: Chromium oxide; 5-chloro-2-methyl-3-(2h)-isothiazolone mixture with 2-methyl-3-(2h)-isothiazolone.

Precautions: Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.[1]

For *in vitro* diagnostic use

Safety Data Sheets (SDS/MSDS) can be found at www.siemens.com/healthcare

Reagent Preparation: Hydrating, Mixing and diluting are automatically performed by the instrument.

REAGENT STORAGE & STABILITY:

Store at 2 – 8°C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. Once wells 1-6 have been entered by the instrument, they are stable for 72 hours. Once well 7 has been entered by the instrument, it is stable for 10 days.

SPECIMEN REQUIREMENTS:

Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.[1]

Follow the instructions provided with your specimen collection device for use and processing.

Each laboratory must determine its appropriate sample timing/collection protocol.

Complete clot formation should take place before centrifugation. Specimens should be free of particulate matter.

Specimen:

- patient preparation: no patient preparation required
- specimen type: serum or heparinized plasma
- stability:

  Separated specimens are stable for 8 hours at room temperature

  Separated specimens are stable for 7 days at 2-8^{o} C

  For longer storage, specimens may be frozen at -20^{o} C or colder for up to 6 months.

CONTROLS:

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

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:

Materials required to perform the DGNA test on the Dimension® clinical chemistry system with the separation module or heterogeneous immunoassay module include:

Materials Needed	**Cat. No.**
DGNA Flex® reagent cartridge	DF35A
Drug Calibrator	DC22B
Reaction vessels (required for HM only)	RXV1A
Quality Control material	

Test Steps

Sampling,[f] reagent delivery, mixing, separation, processing and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® system manual.

f. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus dead volume: Precise container filling is not required.

Test Conditions

Test conditions for separation module or heterogeneous immunoassay module:

Cuvette 1/Reaction Vessel

- Sample Size: 30 µL
- Antibody Conjugate Reagent Volume: 100 µL
- Ouabain Particle Volume: 75 µL
- Diluent Volume: 115 µL
- Incubation Temperature 42°C

Cuvette 2/Cuvette

- Transfer Volume: 60 µL
- Substrate Reagent Volume: 175 µL
- Diluent Volume: 165 µL
- Test Temperature: 37°C
- Wavelength: 577 and 700 nm
- Type of Measurement: bichromatic rate

Calibration

The following information should be considered when calibrating the DGNA method (also see Appendix B):

Assay Range:	0.20–5.00 ng/mL [0.26–6.41 nmol/L]
Calibration Material:	Drug Calibrator (Cat. No. DC22B).
Suggested Calibration Levels:	0.00, 0.60, 1.20, 2.50, 5.00 ng/mL [0.00, 0.77, 1.54, 3.20, 6.41 nmol/L]
Calibration Scheme:	Five levels in duplicate
Calibration Frequency:	Every new reagent cartridge lot Every month for any one lot For each new lot of Flex reagent cartridges After major maintenance or service, if indicated by quality control results As indicated in laboratory quality control procedures When required by government regulations
Assigned Coefficients:	C0 1.0 C1 108.0 C2 –2.0 C3 1.77 C4 0.5

Back-up Testing :
Refer to Brown Clinic Backup Policy

EXPECTED VALUES:

Therapeutic digoxin concentrations vary significantly, depending on the individual. A range of 0.90–2.00 ng/mL [1.15–2.56 nmol/L] includes effective serum concentrations for many patients; however, some individuals are best treated at concentrations outside this range. Concentrations greater than 2.00 ng/mL [2.56 nmol/L] are often associated with toxic symptoms.[4,5]

REPORTING:

The instrument automatically calculates and prints the concentration of digoxin in ng/mL [nmol/L] using the calculation scheme illustrated in your Dimension® system manual.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

LIMITATIONS:

DGNA results less than 0.20 ng/mL [0.26 nmol/L] should be reported as 'less than 0.20 ng/mL [0.26nmol/L]" instead of the numerical value. Results in excess of 5.00 ng/mL [6.41 nmol/L] should be repeated.

Manual Dilution:	Dilute the sample with a digoxin-free serum or Level 1 (0 ng/ml) of Drug Calibrator to produce a sample concentration within the assay range. Enter dilution factor. Reassay. Resulting readout is corrected for dilution.
Autodilution (AD):	Autodilution is not recommended for this method.

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® system manual.

Analytical Specificity

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:

DGNA Flex® reagent cartridge insert sheet PN 717036.001 Issue Date 2015-01-30 Rev. G

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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	Changed instrumentation to EXL200
5/8/15	Amy Harms	Updated information to package insert dated 8/31/2011
8-27-15	Sam Legg	Added Risk and Safety per updated package insert dated 01/30/2015
10/18/17	Heather Hall	Modified backup process