CEDIA™ Digitoxin Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 100004 (17 mL, 13 mL Kit)

Intended Use

The CEDIA™ Digitoxin Assay is an in vitro diagnostic medical device intended for the quantitative determination of digitoxin in human serum.

Summary and Explanation of the Test

Digitoxin is a naturally occurring cardiac glycoside that is used to treat congestive heart failure and atrial fibrillation. Therapeutically, the major pharmacodynamic property of digitoxin is to increase the force of myocardial contractions. It is also known to slow the ventricular rate in cases of atrial fibrillation.

Studies suggest up to 23% of all hospitalized patients treated with digitoxin experienced some degree of toxicity and the mortality rate among toxic patients was more than twice that of nontoxic patients.[1] However, there is a large variability of response among individuals with regard to whether the drug concentration is effective or toxic.

As with most drugs, monitoring serum digitoxin levels should be combined with other clinical data to provide the physician with useful information to aid in adjusting patient dosage, achieving optimal therapeutic effect, while avoiding both sub-therapeutic and harmful toxic drug levels.

The CEDIA Digitoxin assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[2]

The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments i.e., enzyme acceptor (EA) and enzyme donor (ED). These fragments spontaneously reassociate to form active enzyme which, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, digitoxin in the sample competes with the digitoxin-conjugated inactive ED fragment of β-galactosidase for antibody binding sites. If digitoxin is present in the sample, it binds to the digitoxin-specific antibodies, leaving the inactive ED fragments free to recombine with the EA fragments forming active enzyme. If digitoxin is not present in the sample, antibody binds to the digitoxin-conjugated inactive ED fragment, inhibiting the reassociation of the ED fragment with the EA fragment. Thus no active enzyme is formed. Digitoxin concentrations in the sample are directly proportional to the amount of active enzyme formed as monitored by the hydrolysis of chlorophenol red-β-galactopyranoside.

Reagents

- **1 EA Reconstitution Buffer:** Contains HEPES Buffer, stabilizer and preservative, (17 mL).
- **1a EA Reagent:** Contains Enzyme acceptor, (0.28 g/L); anti-digitoxin (sheep) antibodies, (8 mg/L).
- **2 ED Reconstitution Buffer:** Contains HEPES Buffer.
- **2a ED Reagent:** Contains Enzyme donor conjugated to digitoxin, (3.12 µg/L); CPR-β-D-galactopyranoside, (2.21 g/L); donkey anti-sheep antibody, (0.8 g/L).
- **3 Low Calibrator:** Contains normal human serum, (2 mL).
- **4 High Calibrator:** Contains digitoxin in normal human serum, (2 mL).

⚠ Precautions and Warnings

DANGER: Digitoxin powder reagents contain ≤31% bovine serum albumin (BSA), ≤19% Sodium phosphate, dibasic, anhydrous, ≤12% Sodium phosphate, monobasic, ≤10% Donkey serum, ≤1.0% Sodium azide and ≤9% Drug specific antibody (sheep). Digitoxin liquid reagents contain ≤3% Ethylene glycol, ≤0.2% Sodium azide and ≤0.1% Sodium lauroylsarcosinate. Digitoxin controls contain ≤97% human source material and ≤1.3% Sodium azide.
H302 - Harmful if swallowed.
H315 - Causes skin irritation.
H317 - May cause allergic skin reaction.
H319 - Causes serious eye irritation.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H412 - Harmful to aquatic life with long lasting effects.
EUH032 - Contact with acids liberates very toxic gas.

Avoid breathing mist or vapor. Do not eat, drink or smoke when using this product. Avoid release to the environment. Wash hands thoroughly after handling. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. IF SWALLOWED: Call a Poison Center or doctor/physician if you feel unwell. Rinse mouth. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If skin irritation or rash occurs: Get medical advice/attention. If eye irritation persists: Get medical advice/attention. If experiencing respiratory symptoms: call a POISON CENTER or doctor/physician. Take off contaminated clothing and wash before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

The reagents contain sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide buildup. Clean exposed metal surfaces with 10% sodium hydroxide.

☣ Materials of human origin have been tested for HIV 1 and 2, hepatitis B and hepatitis C infections. The findings were negative. However, as no testing method can rule out the risk of potential infection with absolute certainty, the materials must be handled. They are potentially infectious.

Reagent Preparation and Storage

Remove the kit from refrigerated storage immediately prior to preparation of the solutions.

Prepare the solutions in the following order to minimize possible contamination.

R2 Enzyme donor solution: Connect Bottle 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 10 minutes at 15-25°C. Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage and let stand 90 minutes before use.

R1 Enzyme acceptor solution: Connect Bottle 1a (EA Reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 10 minutes at 15-25°C. Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage and let stand 90 minutes before use.

Calibrators: Ready for use. Mix by gentle inversion before use. Avoid the formation of foam.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.
NOTE 2: Avoid cross-contamination of reagents by matching reagent caps to the proper reagent bottle. The R2 Working Solution (Enzyme Donor) should be yellow-orange in color. A red or purple-red color indicates that the reagent has been contaminated and must be discarded.
NOTE 3: The R1 and R2 Working Solutions must be at the reagent compartment temperature of the analyzer before performing the assay.
NOTE 4: To ensure reconstituted EA solution stability, protect from prolonged, continuous exposure to bright light.

Store reagents at 2-8°C. **DO NOT FREEZE.** For stability of the unopened components, refer to the box or bottle labels for the expiration date.

R1 Solution: 30 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 30 days refrigerated on analyzer or at 2-8°C.
Calibrators: up to the expiration date at 2-8°C.

In the case of accidental spill, clean and dispose of materials according to your laboratory's SOP, local and state regulations.

In the case of damaged packaging on arrival, contact your technical support representative (Refer to the back page of this PI).

Specimen Collection and Handling

Serum or plasma (K heparin, Na heparin, Li heparin, and EDTA) samples are suitable for use in the assay. Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the sample from the time it is collected until the time it is assayed. Centrifuge specimens containing particulate matter. Cap samples, store at 2-8°C and assay within 24 hours after collection. If the assay cannot be performed within 24 hours, or if the sample is to be shipped, cap the sample and keep it frozen. Samples can be stored at -20°C for up to 3 months for prolong storage. **Handle all patient samples as if they were potentially infectious.**

Assay Procedure

Chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymtic rates and timing the reaction accurately can be used to perform this assay. Application sheets with specific instrument parameters are available from Microgenics.

NOTE: If the bar code is not read by the analyzer, the numerical sequence on the bar code label can be entered manually via the keyboard.

Quality Control and Calibration[3]

2-Point calibration is recommended

- as blank calibration every 24 hours
- after reagent bottle change
- after reagent lot change
- as required following quality control procedures

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters. Contact Customer Technical Support for further assistance. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Limitations

1. Samples from patients using Uzara® for treatment of diarrhea may cause falsely elevated digitoxin results.
2. The incidence of patients with antibodies to E. coli β-galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high digitoxin results that do not fit the clinical profile.

Results and Expected Values

CEDIA Digitoxin Assay is designed to quantitate patient samples between 3 ng/mL and 50 ng/mL. Specimen giving values below 3 ng/mL should be reported as < 3 ng/mL. Specimen quantitating greater than 50 ng/mL can be reported as > 50 ng/mL or diluted one part sample with one part Digitoxin free sample and reassayed. The value obtained on reassay should be derived as follows:

Actual value = 2 x diluted value

Use the following conversion factor to convert ng/mL to nmol/L:

ng/mL x 1.3 = nmol/L

nmol/L x 0.77 = ng/mL

Therapeutic effectiveness in many patients is not obtained until digitoxin concentrations reach 25 or 30 ng/mL.[4] Conversely, serum digitoxin levels greater than 30 ng/mL are usually considered toxic. However, toxicity may also occur at lower levels.[5] Therefore, for diagnostic purpose, digitoxin results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Specific Performance Characteristics

The following specific performance characteristics were determined using a Hitachi system.[6] Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using controls in an internal protocol:

	Within run (n = 20)			Between day (n = 17)		
Sample	Mean ng/mL	SD ng/mL	% CV	Mean ng/mL	SD ng/mL	% CV
Control 1	10.07	0.70	6.95	9.48	1.12	11.81
Control 2	16.66	0.66	3.96	18.21	1.60	8.79
Control 3	44.02	0.85	1.93	45.46	1.39	3.06

Method Comparison

A comparison using the CEDIA Digitoxin Assay (y) with a commercial radioimmunoassay (x) gave the following correlation (ng/mL):

Linear regression

y = -0.27 + 0.99 x
r = 0.96
SEE = 2.67

Number of samples measured: 137
The sample concentrations were between 3.1 and 49.0 ng/mL.

Linearity

A high sample was diluted with the CEDIA Digitoxin Low Calibrator. The percent recovery was then determined by dividing the assayed value by the expected value.

% High Sample	Expected Value (ng/mL)	Assayed Value (ng/mL)	% Recovery
100	-	34.83	100.0
75	26.12	26.93	103.1
50	17.42	18.30	105.1
25	8.71	9.35	107.3

Recovery

Digitoxin was added to a low patient sample. The percent recovery was then determined by dividing the assayed value by the expected value.

Digitoxin Added (ng/mL)	Expected Value (ng/mL)	Assayed Value (ng/mL)	% Recovery
0	-	9.03	-
9.57	18.60	19.25	103.5
19.14	28.17	28.39	100.8
28.70	37.73	38.68	103.0

Specificity

The following compounds were tested for cross-reactivity.

Compound	% Cross-reactivity
Deslanoside	3.8
Digitoxigenin	217.4
Digitoxigenin-bisdigitoxiside	138.9
Digitoxigenin-monodigitoxiside	177.3
Digoxigenin	3.9
Digoxin	5.3
Compound	**% Cross-reactivity**
Dihydrodigoxin	< 0.1
Furosemide	< 0.1
Gitalin	34.7
Ouabain	1.9
Prednisolone	< 0.1
Prednisone	< 0.1
Progesterone	< 0.1
Quinidine (Free Base)	< 0.1
Quinidine (HCl)	< 0.1
Spironolactone	< 0.1
Testosterone	< 0.1
Theophylline	< 0.1

No interference was found in CEDIA Digitoxin assay with:

Substance	Concentration
Bilirubin	≤ 60 mg/dL
Hemoglobin	≤ 1000 mg/dL
Triglyceride	≤ 1000 mg/dL

Sensitivity

The minimum detectable concentration of the CEDIA Digitoxin Assay is 1.7 ng/mL.

References

1. Beller GA, Smith TW, Abelmann WH, Haber E, Hood Jr. WB. Digitalis Intoxication, A Prospective Clinical Study with Serum Level Correlations, The New England Journal of Medicine 1971; 284(18): 989-997.
2. Henderson DR, Friedman SB, Harris JD, Manning WB, Zoccoli MA. CEDIA, A New Homogeneous Immunoassay System. Clin Chem. 1986; 32(9): 1637-1641.
3. Data on traceability are on file at Microgenics Corporation.
4. Smith TW. Radioimmunoassay for Serum Digitoxin Concentration: Methodology and Clinical Experience, The Journal of Pharmacology and Experimental Therapeutics 1970;175(2): 352-360.
5. Smith TW, and Haber E. The Clinical Value of Serum Digitalis Glycoside Concentrations in the Evaluation of Drug Toxicity, Annals New York Academy of Sciences 1971;179: 322-337.
6. Data on file at Microgenics Corporation, part of Thermo Fisher Scientific.

Glossary:

http://www.thermofisher.com/symbols-glossary

Microgenics Corporation
46500 Kato Road
Fremont, CA 94538 USA
US Customer and
Technical Support:
1-800-232-3342

EC REP
B·R·A·H·M·S GmbH
Neuendorfstrasse 25
16761 Hennigsdorf, Germany

For insert updates go to:
www.thermofisher.com/diagnostics

Other countries:
Please contact your local Thermo Fisher Scientific representative.

10003625-10-EN
2020 06

thermo scientific