Datasheet for ABIN996907

Digoxin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Digoxin (DIG)
Reactivity:	Human
Method Type:	Competition ELISA
Detection Range:	0.25-4.0 ng/mL
Minimum Detection Limit:	0.25 ng/mL
Application:	ELISA

Product Details

Purpose:	The Quantitative Determination of Digoxin Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay,Colorimetric.
Sample Type:	Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	96%
Sensitivity:	0.05 ng /mL
Material not included:	1. Pipette capable of delivering 25, 50 & 100 mL volumes with a precision of better than 1.5 %. 2. Dispenser(s) for repetitive deliveries of 1. 00 mL and 1. 50 mL volumes with a precision of better than 1. %. 3. Microplate washers or a squeeze bottle (optional). 4. Microplate Reader with 405 nm and 620 nm wavelength absorbance capability.

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5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or micro plate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer
9. Quality control materials

Target Details

Target:	Digoxin (DIG)
Alternative Name:	Digoxin (DIG Products)
Target Type:	Chemical
Background:	The clinical usefulness of the measurement of serum digoxin (DIG) is due to its low therapeutic ratio, a very small difference exists between therapeutic and toxic tissue levels. In addition, individuals may vary in their response to digoxin with an apparent increase in susceptibility to toxicity with age. The action of digoxin is to increase the force and velocity of myocardial contraction. This is necessary in the treatment of congestive heart failure and arrhythmias such as atrial fibrillation and atrial flutter. The myocardial concentrations of digoxin to serum levels remain relatively constant during normal renal function. This distribution ratio of digoxin is approximately 29 to 1 between the heart and serum. Thus, monitoring digoxin therapy by measurement of serum levels is feasible from the pharmacological standpoint, since serum levels are related to tissue levels following post-absorption equilibration. A practical and sensitive method of digoxin quantitation in serum is by enzyme immunoassay. This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-digoxin conjugate is added, then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native digoxin for a limited number of antibody combining sites immobilized on the well. After the completion of the required incubation period, the antibody bound enzyme-digoxin conjugate is separated from the unbound enzyme-digoxin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known digoxin concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with digoxin concentration.

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Comment:

Quality Control:

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained - follow the performance of the supplied reagents. Pertinent statistical methods should be employed - ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used - determine the reason for the variations.

Limitations of procedure: Assay Performance It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten (10) minutes - avoid assay drift. If more than one (1) plate is used, it is recommended - repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the end reagent. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence - eliminate any time deviation during reaction. Sample(s) that are contaminated microbiologically should not be used in the assay. Highly lipemeic or hemolysed specimen(s) should similarly not be used. Plate readers measure vertically. Do not touch the bottom of the wells. Failure - remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

Interpretation

1. Certain disease states are known - increase a patient's susceptibility - digoxin toxicity (4). The following are examples of such disease states. a. Hypokalaemia b. Hypothyroidism c. Renal Failure d. Advance Heart Disease

2. A number of researchers have reported relatively high serum digoxin levels in infants. However, digoxin treated-children older than two years of age demonstrate serum digoxin levels more closely resembling adult values (3).

3. Patients receiving simultaneous quinidine and digoxin therapy should be monitored closely (5). Serum digoxin levels may rise - greater than twice the stabilized level within 24 hours after initiation of quinidine therapy and may remain higher for several days.

4. Patients receiving the diuretic furosemide may not display digoxin values that correspond - the Clinical picture (6). When furosemide and digitalis preparations are used concurrently, monitoring patients is desirable (7). TABLE 2 Within Assay Precision (Values in ng/mL) Sample N X sigma C.V. Low 12 0.48 0.04 9.0 % Normal 12 1.67 0.11 6.6 % High 12 3.14 0.16 5.0 % TABLE 3 Between Assay Precision* (Values in ng/mL) Sample N X sigma C.V. Low 10 0.51 0.05 9.8 % Normal 10 1.62 0.13 8.0 % High 10 3.32 0.22 6.6 % *As measured in ten experiments in

	duplicate.
Sample Volume:	25 µL
Assay Time:	1 h
Plate:	Pre-coated
Reagent Preparation:	Preparation Wash Buffer Dilute contents of Wash Solution to 1000 mL with distilled or deionized water in a suitable storage container. Store at room temperature (20-27 °C) for up to 60 days. Working substrate solution - Stable for 1 year Pour the contents of the amber vial labeled as solution "A" into the clear vial labeled solution "B" Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8 °C *Note:* Do not use the working substrate if it looks blue.
Sample Preparation:	Collect sample(s) by venipuncture in ten (10) mL silicone evacuated tube(s) or evacuated tube(s) containing EDTA or heparin. The usual precautions in the collection of venipuncture samples should be observed. Separate the red blood cells by centrifugation. Use serum or plasma for the total DIG procedure. Specimen(s) may be refrigerated at 2-8 °C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. when assayed in duplicate, 0.05 mL of the specimen is required. The cross-reactivity of the Digoxin antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of digoxin needed to displace the same amount of tracer Substance Cross Reactivity Digoxin 1.000 Digitoxin 0.019 Digitoxigenin 0.017 Lanatoside A 0.016 Ouabain 0.001 Spirnolactone 0.001 Prednisone 0.001 Pregnenolone 0.001 Digitoxose 0.001 Di-Acetyldigoxin, beta-Methyldigoxin, a-Acetyldigoxin completely cross react in the assay.
Assay Procedure:	Before proceeding with the assay, bring all reagents, serum references and controls to room temperature. 1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 °C. 2. Pipette 0.025 mL (25 µL) of the appropriate serum reference, control or specimen into the assigned well. 3. Add 0.050 mL (50 µL) of Digoxin-Enzyme Reagent to all the wells. IAGNOSTICS 4. Swirl the microplate gently for 20-30 seconds to mix and cover. 5. Add 0.050 mL (50 µL) Digoxin Biotin Reagent to all wells.

6. Swirl the microplate gently for 20-30 seconds to mix and cover.

7. Cover and Incubate 30 minutes at room temperature.

8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

9. Add 350 µL of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

10. Add 0.100 mL (100µ^ of working substrate solution to (see reagent preparation section).Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER THE SUBSTRATE ADDITION.

11. Incubate at room temperature for thirty (15) minutes.

12. Add 0.050 mL (50 µL of end reagent to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 405 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a microplate reader. The results should be read within five (5) minutes of adding the end reagent.

Note: For re-assaying specimens with concentrations greater than 4 ng/mL, pipet 12 µL of the specimen and 12 µL of the 0 serum reference into the sample well. Multiply the readout value by 2 to obtain the digoxin concentration.

Calculation of Results:	A dose response curve is used to ascertain the concentration of digoxin in unknown specimens. 1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1. 2. Plot the absorbance for each duplicate serum reference versus the corresponding DIG concentration in ng/mL on linear graph paper (do not average the duplicates of the serum references before plotting). Draw the best-fit curve through the plotted points. 3. To determine the concentration of digoxin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the

average absorbance (0.884) intersects the standard curve at (

1.56 ng/mL) digoxin concentration (See Figure 1).

EXAMPLE 1

Sample I.D. Well Number Abs (A) Mean Abs (B) Value (ng/mL) Cal A A1

2.572

2.548 0 B1

2.524 Cal B C1

2.090

2.086 0.25 D1

2.083 Cal C E1

1.738

1.705 0.5 F1

1.671 Cal D G1

1.250

1.255

1.00 H1

1.260 Cal E A2 0.816 0.810

2.00 B2 0.805 Cal F C2 0.474 0.470

4.00 D2 0.467 Patient E2 0.867 0.878

1.56 The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a standard curve prepared with each assay. A

1. 2 3 Q.C. PARAMETERS In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/dL should be >

1.

3.

2. Four out of six quality control pools should be within the established ranges.

A. Precision The within and between assay precision of the DIG DAI ELISA test System were determined by analyses on three different levels of pool control sera. The number (N), mean values (X), standard deviation (sigma) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table

3.

The usual therapeutic range of digoxin in adults is 0.5-

2.0 ng/mL. However, there is an overlap of serum digoxin concentrations in groups of patients with and without clinical toxicity. A significant number of non-toxic patients have serum

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	concentrations greater than 2.0 ng/mL and a correspondingly significant number of toxic patients have serum values in the range of 1.4- 2.0 ng/mL (8). Also, patients with supraventricular arrhythmias may require higher doses to control their cardiac rate: these patients' digoxin concentrations range from 2.0-4.0 ng/mL without clinical toxicity. For these reasons, the physician should make a definite clinical diagnosis after all clinical and laboratory findings have been evaluated.
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Expiry Date:	18 months